

# **AMYLOID ANGIOPATHY IN HEREDITARY GELSOLIN AMYLOIDOSIS**

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Academic dissertation

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*To my mom*

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## List of original publications

This thesis is based on the following original publications, which are referred to as Roman numerals I-III in the text:

- I      **Koskelainen S**, Pihlmaa T, Suominen S, Zhao F, Salo T, Risteli J, Baumann M, Kalimo H, Kiuru-Enari S. Gelsolin amyloid angiopathy causes severe disruption of the arterial wall. *APMIS* 2016; 124(8):639-48.
  
- II     **Koskelainen S**, Zhao F, Kalimo H, Baumann M, Kiuru-Enari S. Severe elastolysis in hereditary gelsolin (AGel) amyloidosis. *Amyloid* 2020; 27(2):81-88.
  
- III    **Koskelainen S\***, Juusela P\*, Nieminen A, Baumann M, Salo T, Risteli J, Uitto VJ, Kiuru-Enari S. Gelsolin c.640G>A mutation in vascular smooth muscle cells and oral fibroblasts of hereditary gelsolin (AGel) amyloidosis patients. *Amyloid*, submitted.  
\*The authors contributed equally to this work.

In addition, unpublished data is presented.

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## Abbreviations

2D-UPLC-MS/MS	two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry
$\alpha$ 1-PDX	$\alpha$ 1-antitrypsin Portland
$\alpha$ -SMA	$\alpha$ -smooth muscle actin
AA	amyloid A
AANF	amyloid atrial natriuretic factor
AApoAI	amyloid apolipoprotein AI
A $\beta$	amyloid $\beta$
A $\beta$ 2M	amyloid $\beta$ 2-microglobulin
ABAD	amyloid $\beta$ -peptide-binding alcohol dehydrogenase
ABP	actin-binding protein
ABri	amyloid BriPP
ACys	amyloid Cystatin C
ADan	amyloid DanPP
AD	Alzheimer's disease
ADH	alcohol dehydrogenase
AEC	3-amino-9-ethylcarbazole
AGel	amyloid gelsolin
AH	amyloid immunoglobulin heavy chain
AL	amyloid immunoglobulin light chain
ALys	amyloid lysozyme
AMed	amyloid medin
ANF	atrial natriuretic factor
ApoAI	apolipoprotein AI
ApoE	apolipoprotein E
APrP	amyloid prion protein
ATTR	amyloid transthyretin
$\beta$ 2M	$\beta$ 2-microglobulin
CAA	cerebral amyloid angiopathy
CCD	charge-coupled device



CLD	corneal lattice dystrophy (also CLA corneal lattice amyloidosis)
DAB	diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DBP	vitamin D-binding protein
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
FAF	familial amyloidosis Finnish type
FBD	familial British dementia
FDD	familial Danish dementia
FIN-GAR	Finnish Gelsolin Amyloidosis Patient Registry
GAG	glycosaminoglycan
HCCAA	hereditary cystatin C amyloid angiopathy
HCHWA-I	hereditary cerebral hemorrhage with amyloidosis, Icelandic type
HGA	hereditary gelsolin amyloidosis
HLA	human leukocyte antigen
HUH	Helsinki University Hospital
IAA	isolated atrial amyloidosis
ICTP	C-terminal telopeptide of type I collagen
IHC	immunohistochemistry
IIINTP	N-terminal telopeptide of type III collagen
kDa	kilodalton (a unit of molecular mass)
LOX	lysyl oxidase
MMP	matrix metalloproteinase
PAI	plasminogen activator inhibitor
PICH	primary intracerebral hemorrhage
PIIINP	N-terminal propeptide of type III procollagen
PINP	N-terminal propeptide of type I procollagen
PIP <sub>2</sub>	phosphatidylinositol 4,5-bisphosphate
PLGA	poly lactic-co-glycolic acid
PMA	phorbol 12-myristate 13-acetate
PrP	prion protein
RIA	radioimmunoassay

SAA	serum amyloid A
SAP	serum amyloid P component
TGFβ	transforming growth factor beta
TIMP	tissue inhibitor of metalloproteinase
TEM	transmission electron microscope
TTR	transthyretin
vAGel	variant gelsolin
VSMC	vascular smooth muscle cell

## Abstract

Hereditary gelsolin amyloidosis (HGA), also known as AGel amyloidosis and Meretoja disease, is a dominantly inherited systemic disease that belongs to the Finnish disease heritage. HGA is mostly found in Finland but nowadays also reported worldwide.

The disease is caused by a c.640G>A/T point mutation in the *GSN* gene coding for gelsolin which further results in the mutation p.D214N or p.D214Y on the protein level. Due to these mutations, variant gelsolin is misfolded and undergoes abnormal enzymatic cleavages by furin and matrix metalloproteinase 14 (MMP-14) leading to gelsolin amyloid (AGel) formation. Gelsolin is expressed in two forms of which the extracellular one has been indicated to be the source for the misfolded form. Gelsolin has several functions of which the most important is actin fibril remodeling intra- and extracellularly.

HGA is characterized mainly by ophthalmological, neurological and dermatological manifestations. However, HGA patients can also have cardiovascular, hemorrhagic and potentially vascularly induced neurological problems. AGel amyloid fibrils accumulate extracellularly at the basal lamina of epithelial and muscle cells and alongside elastic fibres. Thus, amyloid angiopathy is encountered in nearly every organ. AGel deposition associates with elastic fibre degradation leading to severe clinical manifestations, such as cutis laxa and angiopathic complications.

The aim of this study was to characterise the pathological changes of AGel amyloid angiopathy in small arteries, to elucidate pathomechanisms of amyloid related elastolysis, and to investigate the effects of variant gelsolin in vascular smooth muscle cells from the HGA patients.

To characterise the pathological changes of AGel amyloid angiopathy, we performed histological, immunohistochemical and transmission electron microscopic (TEM) analyses on facial temporal artery branches. This study revealed major pathological changes in arteries: disruption of the *tunica media*, disorganization of vascular smooth muscle cells, and accumulation of AGel fibrils in arterial walls, where they associate strongly with the *lamina elastica interna*, which becomes fragmented and diminished. We also provide evidence of abnormal accumulation and localization of collagen types I and III and an increase of collagen type I degradation product in the *tunica media*.

To elucidate pathomechanisms of amyloid related elastolysis, we analysed elastic fibre pathology in dermal and vascular tissue and plasma samples from HGA patients and control subjects by TEM, immunohistochemistry and enzyme-linked immunosorbent assay (ELISA). In addition to the morphological examination, we also studied the roles of MMP-2, -7, -9, -12 and -14, TIMP-1 and TGF $\beta$ . We found massive accumulation of amyloid fibrils along elastic fibres as well as fragmentation and loss of elastic fibres in all dermal and vascular samples of HGA patients. Fibrils of distinct types formed a fibrous matrix. The degradation pattern of elastic fibres in HGA patients was different from the age-related degradation in controls. The elastin of elastic fibres in HGA patients was also remarkably decreased compared to controls. Interestingly, MMP-9 was expressed at lower and TGF $\beta$  at higher levels in HGA patients than in controls.

To investigate the effects of gelsolin amyloidosis in vascular smooth muscle cells (VSMCs) we established unique cell lines from the HGA patients. As the c.640G>A mutation is located in the actin regulatory site of gelsolin, we speculated that this could impair cytosolic functions of gelsolin. We therefore conducted cell studies examining actin cytoskeleton morphology, cytosolic gelsolin distribution, migration, and collagen type I metabolism in VSMCs. We also treated the cells with phorbol 12-myristate 13-acetate (PMA) and staurosporine, regulators of protein kinase C, but the HGA patient and control cell lines did not show significant differences in any of these study settings. Also, in TEM analyses VSMCs in arteries appeared to be morphologically and semi-quantitatively normal, only their basal lamina was often thickened.

According to this study the AGel amyloid angiopathy in HGA results in severe disruption of arterial walls, characterized by prominent AGel deposition, collagen derangement and severe elastolysis, which may be responsible for several, particularly hemorrhagic, disease manifestations in HGA. The accumulation of AGel fibrils with severe elastolysis characterizes both dermal and vascular tissues. The unaltered cytoskeletal actin-gelsolin interactions in studied cells imply that HGA results rather from a toxic gain-of-function than loss-of-function mechanism. Furthermore, we found two potential biomarkers to be used as diagnostic markers in evaluating the progression of HGA in patients. The first one, alcohol dehydrogenase 1B (ADH1B), was found in two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS) proteomic analyses of VCMs, and the second one, the C68 fragment of variant gelsolin, from HGA patient tear fluid by Western blot analysis,

providing a non-invasive way to measure the level of misfragmentation of gelsolin in HGA patients.

# Introduction

I have been involved with hereditary gelsolin amyloidosis (HGA) all my life. I was born in Kymenlaakso which is one of the areas where HGA is highly represented. The prevalence of HGA in Kymenlaakso was estimated in 1970s to be as high as 1:1 040 (Meretoja, 1973). There are many families that carry this hereditary disease, including my own.

Finland has been an optimal environment for development of rare hereditary diseases due to the national and regional isolation. HGA is one member of the Finnish disease heritage which includes altogether 36 different inherited diseases that are more frequent in Finland than in any other population.

HGA is a systemic amyloidosis that affects many tissues and organs all around the body. In amyloidosis proteins or peptides are self-assembling into  $\beta$ -sheets structures which further form organized amyloid fibrils. These amyloid fibrils accumulate to different tissues depending on the nature of the protein or peptide and other factors that favor fibril formation. The best-known amyloidosis is Alzheimer's disease where amyloid is locally deposited in the brain causing neurodegeneration and severe dementia.

HGA is caused by a mutation in the gelsolin protein. Variant gelsolin undergoes an alternative proteolytic cleavage leading to AGel amyloid formation. AGel accumulates at the basal lamina of many types of epithelial- and muscle cells, as well as peripheral nerves. Deposition of AGel is found in arterial walls in nearly every organ, including the nervous system.

HGA is characterized by ophthalmological, neurological, and dermatological manifestations. Although HGA progresses slowly in heterozygous patients it causes many difficulties like dry and irritable eyes, loose, itching and dry skin, distal paresthesias and manual clumsiness, bilateral facial nerve paresis which can cause severe facial disfigurement, dysarthria and, loss of vision, with severely decreased quality of life. As a possible consequence of amyloid angiopathy, HGA patients get easily superficial bruises and hematomas, they have commonly cardiac diseases and arrhythmias, and the consumption of cardiovascular medication is significantly increased.

I have had a privilege to study HGA both in my masters and doctoral theses. For this study we collected tissue and blood samples from HGA patients and control persons. We wanted to

characterize the pathological changes of AGel amyloid angiopathy, elucidate pathomechanisms of amyloid related elastolysis and investigate the effects of HGA in vascular smooth muscle cells with a unique set of cell lines from the HGA patients.

# Review of the literature

## 1 Amyloid angiopathy

Angiopathy is a general term for different diseases of the blood vessels. In this thesis the focus is on the angiopathies caused by amyloid accumulation in the vessel walls called either amyloid angiopathies or vascular amyloidoses.

### 1.1 Amyloid

In amyloid diseases proteins or peptides are self-assembling into well-organised fibrillar aggregates forming  $\beta$ -sheet structures.  $\beta$ -sheets consist of  $\beta$ -strands which are connected and stabilized by at least two or three interstrand hydrogen bonds.  $\beta$ -sheet structures are known as amyloid or amyloid like fibrils (Sipe, Cohen, 2000). These fibrils are insoluble and cannot be digested enzymatically which makes them pathological as they accumulate intracellularly and/or in the extracellular spaces of organs and tissues. Amyloid formation is generally caused by protein misfolding due to a mutation in the target protein and/or by other surrounding factors like pathological chaperons, metal ions ( $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  or  $\text{Fe}^{3+}$ ) and/or conditions like oxidative stress.

Chaperons and oxidative stress play a major role in amyloid fibril formation. Often late-onset neurodegenerative diseases are developing because chaperons are losing their capacity of regulating the protein folding process. There are also specific pathological chaperons like glycosaminoglycans (GAGs) (Iannuzzi, Irace & Sirangelo, 2015), serum amyloid P component (SAP) (Pepys et al., 1982), apolipoprotein E (ApoE) (Soto et al., 1995, Liu et al., 2017) and collagen fibres (Benseny-Cases et al., 2019) which enable and accelerate amyloid fibril formation.

Amyloid is recognized by specific properties, such as green-yellow birefringence under polarized light after staining with Congo red dye (Puchtler, Sweat & Levine, 1962, Benson et al., 2018). Also, Thioflavin S and T dyes are partially specific for amyloid deposits. Under electron microscopy amyloid fibrils appear like disordered non-branching rods but they can appear even orientated when occurring in massive amounts (Cohen, Calkins, 1959). Amyloid



fibrils are approximately 10 nm in diameter. Isolated amyloid fibrils can be analysed also with X-ray where they show the characteristic cross  $\beta$  diffraction pattern (Eanes, Glenner, 1968). Different types of amyloid proteins are generally characterised using immunohistochemistry where histological tissue samples can be analysed with one or several specific antibodies against amyloidogenic proteins or peptides (Linke, 2012).

## 1.2 Amyloidoses

Amyloidoses are a family of diseases characterised by the extracellular and/or intracellular deposition and accumulation of amyloid fibrils with concomitant destruction of normal tissue structure and function leading to organ dysfunction (Cohen, 1967, LaFerla, Green & Oddo, 2007). Amyloid fibrils can accumulate e.g. in brain and nerves, heart and blood vessels, kidney, liver, spleen, and ocular structures causing different clinical phenotypes like dementia, neuropathy, cardiomyopathy, hypertension, hepatomegaly, proteinuria, renal failure, macroglossia, autonomic dysfunction, ecchymoses, and corneal and vitreous abnormalities (Bustamante, Brito, 2017). More than 30 different proteins causing amyloidosis are known. Table 1 shows all fibril proteins reported to date, listed according to the International Society of Amyloidosis 2018 Nomenclature Guidelines (Benson et al., 2018).

Amyloidoses are categorised in to systemic and localised as well as acquired and hereditary forms. The most common types of systemic amyloidoses (fibril protein in brackets) are light chain amyloidosis (AL), inflammation related serum amyloid A amyloidosis (AA), hemodialysis-related  $\beta$ 2-microglobulin amyloidosis (A $\beta$ 2M), and transthyretin amyloidosis (ATTR) whereas Alzheimer's disease (A $\beta$ ) is the most common and known type of localised amyloidosis. Less than a half of all different amyloidoses known at present are hereditary. These inherited forms of amyloidosis are almost always systemic, where protein misfolding due to a mutation in the amyloid precursor protein is involved. The term familial amyloidosis is also still in use although it is not recommended anymore (Benson et al., 2018). Previously familial amyloidosis was used when the syndrome occurred in a familial setting due to mutations in genes expressing non-amyloid proteins, such as in AA amyloidosis, whereas hereditary amyloidosis were used when there was a mutation in the fibril protein gene itself, like in e.g. ATTR or AGel (Sipe et al., 2016). Amyloidoses were in the past also categorised as primary, secondary and sporadic

amyloidoses but these terms are not in use any longer. Globally the incidence of amyloidosis is estimated at five to nine cases per million patient-years (Real de Asua et al., 2014).

**Table 1.** Amyloid fibril proteins and their precursors in humans. Modified after International Society of Amyloidosis nomenclature committee (Benson et al., 2018).

Amyloid angiopathy known to be involved:				
Fibril protein	Precursor protein	Systemic and/ or localized	Acquired or hereditary	Target organs
ABri	ABriPP, variants	S	H	Central nervous system
ACys	Cystatin C, variants	S	H	Peripheral nervous system, skin
ADan	ADanPP, variants	L	H	Central nervous system
APrP	Prion protein, wild type	L	A	Creutzfeldt-Jakob disease, fatal insomnia
	Prion protein variants	L	H	Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, fatal insomnia
	Prion protein variant	S	H	Peripheral nervous system
<b>A<math>\beta</math></b>	<b>A<math>\beta</math> protein precursor, wild type</b>	<b>L</b>	<b>A</b>	<b>Central nervous system</b>
	<b>A<math>\beta</math> protein precursor, variant</b>	<b>L</b>	<b>H</b>	<b>Central nervous system</b>
AGel	Gelsolin, variants	S	H	Peripheral nervous system, cornea, cutis laxa
ATTR	<b>Transthyretin, wild type</b>	<b>S</b>	<b>A</b>	<b>Heart mainly in males, ligaments, tenosynovium</b>
	<b>Transthyretin, variants</b>	<b>S</b>	<b>H</b>	<b>Peripheral and autonomic nervous system, heart, eye, leptomeninges</b>
AL	Immunoglobulin light chain	S, L	A, H	All organs, usually except central nervous system
AH	Immunoglobulin heavy chain	S, L	A	All organs except central nervous system
AA	(Apo) Serum amyloid A	S	A	All organs except central nervous system
A $\beta$ 2M	$\beta$ 2-Microglobulin, wild type	S	A	Musculoskeletal system
	$\beta$ 2-Microglobulin, variant	S	H	Autonomic nervous system
AApoA1	Apolipoprotein A I, variants	S	H	Heart, liver, kidney, PNS, testis, larynx (C-terminal variants), skin (C-terminal variants)
ALys	Lysozyme, variants	S	H	Kidney
AANF	Atrial natriuretic factor	L	A	Cardiac atria
AMed	Lactadherin	L	A	Senile aortic, media

Amyloid fibril proteins that causes amyloidoses with cerebral amyloid angiopathy (CAA) in **bold**.

Amyloid fibril proteins that causes amyloidoses with systemic or localized amyloid angiopathy in *italics*.

**No amyloid angiopathy known to be involved:**

<b>Fibril protein</b>	<b>Precursor protein</b>	<b>Systemic and/ or localized</b>	<b>Acquired or hereditary</b>	<b>Target organs</b>
AApoAII	Apolipoprotein A II, variants	S	H	Kidney
AApoAIV	Apolipoprotein A IV, wild type	S	A	Kidney medulla and systemic
AApoCII	Apolipoprotein C II, variants	S	H	Kidney
AApoCIII	Apolipoprotein C III, variants	S	H	Kidney
ALECT2	Leukocyte chemotactic factor-2	S	A	Kidney, primarily
AFib	Fibrinogen $\alpha$ , variants	S	H	Kidney, primarily
A $\alpha$ Syn	$\alpha$ -Synuclein	L	A	Central nervous system
ATau	Tau	L	A	Central nervous system
ACal	(Pro)calcitonin	L	A	C-cell thyroid tumors
AIAPP	Islet amyloid polypeptide*	L	A	Islets of Langerhans, insulinomas
APro	Prolactin	L	A	Pituitary prolactinomas, aging pituitary
AIns	Insulin	L	A	Latrogenic, local injection
ASPC	Lung surfactant protein	L	A	Lung
AGal7	Galectin 7	L	A	Skin
ACor	Corneodesmosin	L	A	Cornified epithelia, hair follicles
AKer	Kerato-epithelin	L	A	Cornea, hereditary
ALac	Lactoferrin	L	A	Cornea
AOAAP	Odontogenic ameloblast-associated protein	L	A	Odontogenic tumors
ASem1	Semenogelin 1	L	A	Vesicula seminalis
AEnf	Enfurvitide	L	A	Latrogenic
ACatK	Cathepsin K	L	A	Tumor associated

\* Also called amylin.

## 1.3 Vascular system

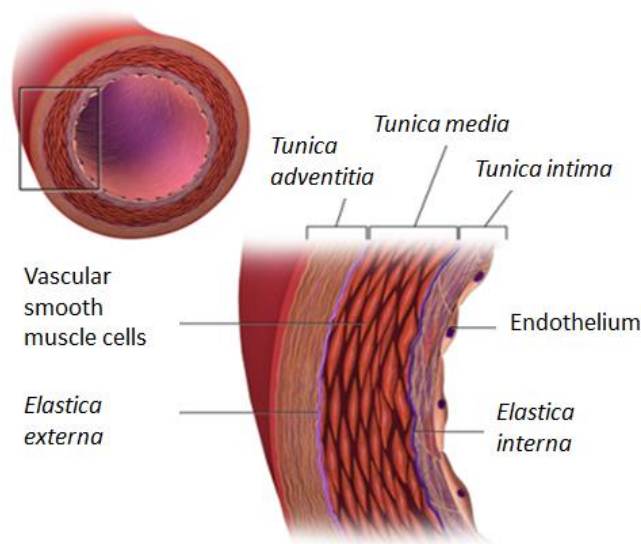
The vascular system includes all vessels of the body from the aorta to arteries, capillaries and veins. Through this system blood streams to different parts and organs in the body. Arteries and their smaller branches called arterioles are the vessels that carry blood away from the heart. In the smallest very thin walled vessels, capillaries, all nutrients and wastes are exchanged between the blood and body tissues. Capillaries connect the arterioles and venules, which enables returning the blood back to the heart through veins.

### 1.3.1 Structure of arteries

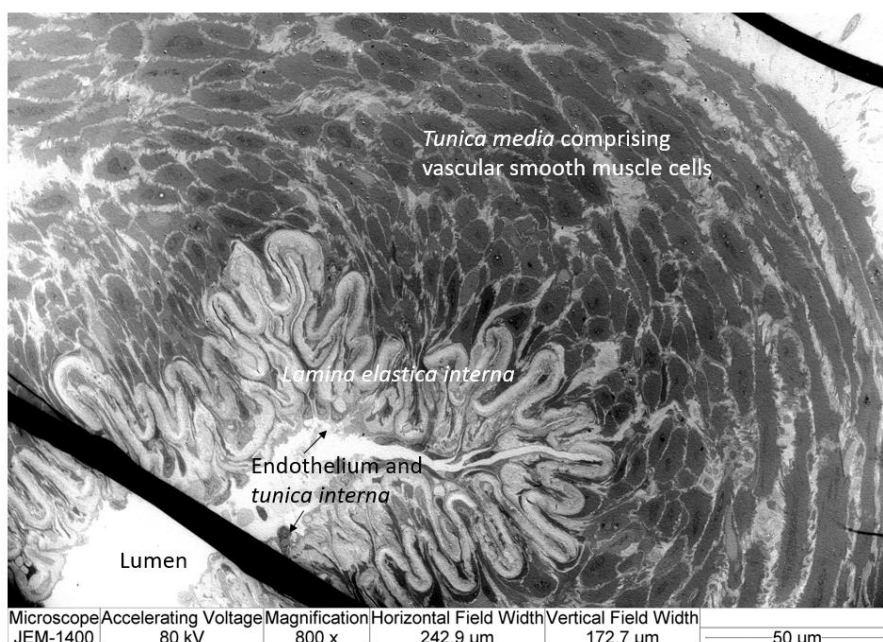
The wall of an artery consists of three layers: 1) the *tunica intima* (the innermost layer), 2) the *tunica media* (the middle coat) 3) the *tunica adventitia* (the outermost layer) (Figure 1 and 2).

In the *tunica intima* the inner surface is lined by a smooth endothelium which is separated from the external layers of the artery by the basal lamina. In the interface of the *tunica intima* and the *tunica media* lies a layer of elastic fibres called the *lamina elastica interna*. The *tunica media* consist of smooth muscle cells, elastic and collagen fibres and other connective tissue components. Between the outermost layer, the *tunica adventitia*, and the *tunica media* there is another layer of elastic fibres, the *lamina elastica externa*. The *tunica adventitia* is composed entirely of connective tissue components made of collagenous and elastic fibres. In larger arteries the *tunica externa* includes also separate blood vessels called the *vasa vasorum* that supply nutrients to these vessels. The smallest blood vessels, arterioles, which represent continuation of arterial branches and lead arterial blood to the capillary bed, have the *tunica intima* but their *tunica media* may contain only a single layer of smooth muscle cells and lack elastic fibres.

Collagen is extracellular insoluble polymeric protein (Bailey, 1978). Out of 28 different collagen types, collagen type I and III are major constituents of the *tunica intima*, *tunica media* and *tunica adventitia*. Also collagen type IV and V are represented in the endothelial basement membrane and basement membranes of smooth muscle cells of the *tunica intima* and *tunica media* (Shekhonin et al., 1985, Ricard-Blum, 2011, Xu, Shi, 2014).



**Figure 1** Schematic structure of an artery wall.



**Figure 2** Structure of an artery wall in arterial sample in TEM analysis.

### 1.3.2 Vascular smooth muscle cells

Muscle cells are divided into three different types: skeletal, cardiac and smooth muscle cells. Smooth muscle cells are present in the walls of many hollow organs like in the bronchial, vascular, gastrointestinal, reproductive and urogenital systems, and also in the eyes. Smooth muscle cells are not striated as the other muscle cell types. In line with cardiac muscle cells, smooth muscle cells are involuntarily controlled, not branched and singly nucleated.

Vascular smooth muscle cells (VSMCs) are one of the main components of the vascular wall. VSMCs regulate the blood volume, flow and blood pressure in the vessels, due to their myosin-actin interactions, by contracting and relaxing in response to vasoactive stimuli (Michel, Li & Lacolley, 2012). VSMCs are located in the *tunica media* where they provide structural integrity and control the diameter of the vessel. Arteries have a greater amount of VSMCs than veins, thus their walls are much thicker.

Mature VSMCs in the vessel wall can be defined as the contractile phenotype. However, VSMCs are sensitive to different physiological or pathological stimuli, such as growth factors, mitogens, inflammatory mediators and mechanical influences, and are able to undergo rapid

changes in their functional and morphological properties at different developmental stages, during vascular repair, and in vascular disease (Owens, 1995, House et al., 2008). VSMCs are very plastic cells as they can acquire a broad spectrum of different phenotypes from quiescent contractile to more migratory, proliferative, synthetic, endocytic, phagocytic, or osteoblastic ones (Michel, Li & Lacolley, 2012). In injury or during development, VSMCs changes to migratory and proliferate phenotype and are then able to accumulate in the intima and synthesize extracellular matrix proteins. This phenotype is called synthetic cell. VSMCs also show considerable differences depending on their position in the arterial tree (large vs. small vessels), their embryologic origin and their organ-dependent microenvironment e.g. in the heart, brain and kidney (Michel, Li & Lacolley, 2012).

### *Smooth muscle $\alpha$ -actin*

For different functionalities and morphological properties VSMCs have evolved a repertoire of appropriate contractile proteins, agonist receptors, ion channels, and signal-transducing molecules (Owens, 1995). The differentiated state of the VSMCs is characterized by e.g. specific contractile proteins and cell surface receptors (Metz, Patterson & Wilson, 2012). One of the contractile proteins, widely used for characterization of VSMCs, is the smooth muscle  $\alpha$ -actin. It is one of eight isoactins expressed in mammalian cells (Vandekerckhove, Weber, 1979, Rubenstein, 1990) and the most abundant of the actin isoforms in a mature fully differentiated VSMC. Smooth muscle  $\alpha$ -actin is also the most abundant protein in smooth muscle cells making up to 40 % of total cell protein and over 70 % of the total actin (Fatigati, Murphy, 1984). Other isoforms expressed by VSMCs are nonmuscle  $\beta$ -actin, nonmuscle  $\gamma$ -actin, and smooth muscle  $\gamma$ -actin (Gabbiani et al., 1981).

### **1.3.3 Vascular smooth muscle cells in amyloidoses**

VSMCs are involved in several vascular diseases due to their complexed signaling system and versatile plasticity (Michel, Li & Lacolley, 2012). They have a major role e.g. in hypertension (Lee et al., 1995) and atherosclerosis (Bennett, Sinha & Owens, 2016) but also in different amyloidoses. VSMCs are suggested to be responsible for  $\beta$ -amyloid deposition in the vascular wall in Alzheimer's disease (Wisniewski, Frackowiak & Mazur-Kolecka, 1995) and synthezitation of the amyloid precursor protein lactadherin in AMed amyloidosis (Haggqvist et al., 1999). Recently, VSMCs have been linked to the amyloid formation of amyloidogenic

immunoglobulin light chains in AL amyloidosis (Vora, Kevil & Herrera, 2017). VSMCs participate in the formation of AL by the intracellular processing of amyloidogenic light chains, which is possible due to their transformation from a smooth muscle to a macrophage phenotype.

#### **1.3.4 Angiopathies in amyloidoses**

Overall, the cardiovascular system is a common target of different amyloidoses, such as AL, ATTR, AANF and AMed related amyloidosis (see table 1 for definitions) (Kholova, Niessen, 2005). Wang et al. excellently summarizes the consequences of amyloid deposition depending on the site in the vasculature system: “If amyloid proteins deposit within the walls of the cerebral vasculature with subsequent aggressive vascular inflammation, it will lead to recurrent hemorrhagic strokes; If they deposit within the walls of the coronary artery, they will lead to angina pectoris, even ischemia cardiomyopathy; If they deposit within the wall of aorta, they will lead to hypertension, atherosclerosis, and even dissecting aneurysm eventually.” (Wang et al., 2017). In addition, peripheral nerve amyloid angiopathy may contribute to sensory and motor nerve injury (Kiuru-Enari et al., 2002, Yamashita et al., 2005).

##### *Cerebral amyloid angiopathies*

The best known type of amyloid angiopathy is cerebral amyloid angiopathy (CAA) which is defined by the deposition of amyloid within the walls of small to medium-sized blood vessels of the brain: leptomeningeal and cortical arteries, arterioles and, less frequently, capillaries and veins (Ghisso, J., Frangione, 2001). The most common manifestation of CAA is cerebral hemorrhage, as primary intracerebral hemorrhage (PICH), but it may also lead to ischemic infarction and dementia (Pezzini et al., 2009). At present CAA is known to associate with deposition of seven amyloid proteins, namely: A $\beta$ , ACys, ABri, ADan, APrP, ATTR and AGel.

CAA has been widely studied and reported in Alzheimer’s disease (AD). AD patients are characterized by the extracellular deposition of amyloid A $\beta$  protein in cerebral parenchymal plaques and blood vessels. A large majority of the patients diagnosed with AD suffer of stroke-like lesions or infarctions, ranging from CAA, degenerative microangiopathy compromising both the endothelium and smooth muscle cells, cerebral infarcts, microinfarction, white matter changes related to small vessel disease and even hemorrhages (Ghisso, J., Frangione, 2001).

Hereditary cystatin C amyloid angiopathy (HCCAA,) also known as hereditary cerebral hemorrhage with amyloidosis of Icelandic type (HCHWA-I), is an autosomal dominant disorder caused by a mutation in the *CST3* gene (Abrahamson et al., 1987). Variant Cystatin C is abundant in cerebrospinal fluid and associated with cerebral hemorrhages, typically in young adult carriers (Snorraddottir et al., 2017). HCCAA is characterized by massive amyloid deposition within small arteries and arterioles of leptomeninges, cerebral cortex, basal ganglia, brainstem and cerebellum. Although the brain involvement is the main feature in HCCAA, amyloid deposits are present also in peripheral tissues such as skin, lymph nodes, spleen, salivary glands, and seminal vesicles. In HCCAA PICH dominates the clinical picture unlike in other CAA-related amyloidosis (Pezzini et al., 2009).

Familial British dementia (FBD), is an autosomal dominant form of CAA caused by a mutation in the single multiexonic gene *BRI2* located on the long arm of chromosome 13 (Ghiso, J. A. et al., 2001). FBD is characterized by progressive dementia, spastic tetraparesis and cerebellar ataxia in the age of 40s-50s (Verbeek, de Waal & Vinters, 2013). Amyloid angiopathy is severe and widespread in the brain and spinal cord with perivascular amyloid plaque formation. However, large intracerebral hemorrhage is a rare manifestation of the disease although the central nervous system is extensively loaded with amyloid (Verbeek, de Waal & Vinters, 2013). The amyloid subunit (ABri) was extracted from FBD brain tissues (Vidal et al., 1999).

Familial Danish dementia (FDD) is an autosomal dominant neurodegenerative disease caused by a mutation in the same gene *BRI2* as in FBD (Ghiso, J., Frangione, 2001). FDD is characterized by the existence of widespread cerebral amyloid angiopathy (CAA) in vessels of the retina and leptomeninges, and in vessels of the central nervous system (Vidal et al., 2009). Extensive amyloid angiopathy is present in the blood vessels of the cerebrum, choroid plexus, cerebellum, spinal cord, and retina. In spite of that, also in FDD the incidence of cerebral hemorrhage is rare (Vidal et al., 2009).

In prion diseases or prionoses the etiology is related to the conversion of the normal prion protein PrP<sup>C</sup> into an infectious and pathogenic form PrP<sup>Sc</sup> (Colby, Prusiner, 2011). The latter form can also accumulate as amyloid in the brain. Prionoses include Creutzfeldt-Jakob disease, kuru, Gerstmann-Sträussler-Scheinker disease and fatal familial insomnia in humans as well as scrapie, bovine spongiform encephalopathy, transmissible mink encephalopathy and chronic wasting disease in animals (Ghetti et al., 1996b, Prusiner, 1998, Collinge, 2001). Familial PrP-CAA is a fatal neurodegenerative disease associated with point mutations of the prion protein



gene (*PRNP*) (Jansen et al., 2010). In PrP-CAA amyloidosis is predominantly vascular in contrary to other prionoses. PrP amyloid fibrils are seen adjacent to and within the vessel wall associated with neurofibrillary lesions (Ghetti et al., 1996a). PrP-CAA has a broad spectrum of clinical presentations where the main signs are ataxia, spastic paraparesis, extrapyramidal signs and dementia.

Transthyretin (TTR) amyloidosis is caused by deposition of wild-type (ATTRwt) or variant (ATTRv) amyloidogenic TTR. ATTRwt amyloidosis has traditionally been called senile systemic amyloidosis and ATTRv amyloidosis has been termed familial TTR amyloidosis (Pitkänen, Westermark & Cornwell, 1984, Koike, Katsuno, 2019). Though wild-type TTR can form amyloid, the hereditary form of ATTR amyloidosis is caused by autosomal dominant mutations in the *TTR* gene. The most common ATTR mutation worldwide is V30M but more than 100 pathogenic *TTR* mutations are reported thus far (Kapoor et al., 2019). The phenotypes and age of onset varies greatly depending of the mutation. The hallmarks in ATTR amyloidosis are peripheral neuropathy and involvement of visceral organs (e.g. kidney and ovaries), whereas central nervous system involvement is atypical. ATTR amyloid deposits are present in the blood vessels in the brain and spinal cord, where small and medium size vessels are most gravely affected (Ghisso, J., Frangione, 2001). In addition to ischaemia, amyloid angiopathy has also been reported to cause ocular problems ATTRv amyloidosis (Kawaji et al., 2005).

Cerebral amyloid angiopathy is found also in HGA (Kiuru, Salonen & Haltia, 1999).

### *Systemic amyloid angiopathies*

Systemic amyloidoses are caused by extracellular deposition of misfolded circulating proteins as amyloid fibrils and affect various vital organs including the vascular system. Several systemic amyloidoses can involve the entire cardiovascular system. These include AL, AH, AA, A $\beta$ 2M, AApoAI and ATTR related amyloidosis (Kholova, Niessen, 2005). Vascular involvement is also reported in ALys (Benyammine et al., 2017) and in AGel (Meretoja, Teppo, 1971, Kiuru, Salonen & Haltia, 1999, Juusela, P. et al., 2009, Pihlmaa et al., 2016, Schmidt et al., 2019) related amyloidosis. Amyloid is deposited in the *tunica media* and *tunica adventitia* causing thickening of the wall. This often leads to obstruction and consequent ischaemia. Vascular involvement is less frequent in acquired amyloidoses, mostly involving small intramyocardial vessels (Kholova, Niessen, 2005).

The most prevalent type of the systemic amyloidoses is light-chain (AL) amyloidosis (Merlini, 2017). This amyloidosis is caused by misfolded monoclonal immunoglobulin light chains produced by plasma cells. Vascular involvement affecting medium to large arterioles and small arteries is actually a very typical feature in light-chain amyloidosis, it is reported in 88–90 % of patients (Eder et al., 2007). Abnormal vascular morphology and endothelial dysfunction is seen in light chain amyloidosis (Modesto et al., 2007). Also, severe pulmonary hypertension without an apparent cardiac or parenchymal lung involvement and portal hypertension has been reported (Eder et al., 2007, Norero et al., 2013). Although cardiac involvement is the main cause of morbidity and mortality in light chain amyloidosis (Merlini, 2017) vascular abnormalities do not appear to be related to it (Modesto et al., 2007).

Similarly to AL, AH-amyloidosis is also caused by misfolded monoclonal immunoglobulin but in this disease amyloid is composed of immunoglobulin heavy chains. Unlike light chain amyloidosis, this is a rare disease with very few cases thus far reported (Picken, 2007, Manabe et al., 2015).

Another common type of systemic amyloidosis is AA amyloidosis. This disease is directly related to inflammations. In AA amyloidosis amyloid deposits are composed mainly of the serum amyloid A (SAA) protein, which is a major acute phase reactant in inflammations (Real de Asua et al., 2014). When an abnormally high plasma concentration of SAA persists for a long time in serum, SAA aggregates into amyloid fibrils. The expression of cytokines, in particular interleukin 6, leads to overexpression of SAA by the liver (Westermarck, Fandrich & Westermarck, 2015). AA amyloidosis is typically found in patients with rheumatoid arthritis, or with familial Mediterranean fever, however only a small number of patients with inflammatory conditions will eventually develop amyloidosis (Real de Asua et al., 2014). AA amyloidosis affects various vital organs, like kidney, and it may also cause an increased risk of developing coronary artery diseases (Bulut et al., 2016).

$\beta$ 2-microglobulin ( $\beta$ 2M) amyloidosis is known as dialysis-related amyloidosis. Often this amyloidosis is a consequence of a long-term dialytic therapy but it can be found even in patients with a chronic renal failure before starting the dialysis (Kaneko, Yamagata, 2018). This suggests that the cause for  $\beta$ 2M amyloid formation is accumulation of  $\beta$ 2M or some  $\beta$ 2M-associated molecules in the body due to different reasons.  $\beta$ 2M is the light chain of class I human leukocyte antigen (HLA) on all nucleated cells. The expression of  $\beta$ 2M is normally constant, but increases in infection, inflammation, and lymphoproliferative disorders (Kaneko,

Yamagata, 2018). A $\beta$ 2M is deposited predominantly in the bones, tendons and joints but also vascular and endocardial involvement has been described (Kholova, Niessen, 2005).  $\beta$ 2M amyloidosis can also be caused by a variant  $\beta$ 2M which is inherited in autosomal dominant manner. Unlike patients with dialysis-related amyloidosis, these patients have normal renal function and normal rates of circulating  $\beta$ 2M (Valleix et al., 2012). However, the variant  $\beta$ 2M is unstable and remarkably fibrillogenic *in vitro* under physiological conditions (Valleix et al., 2012).

Apolipoprotein AI (ApoAI) amyloidosis is a hereditary systemic disease caused by germline mutations in *APOA1* gene. ApoAI is involved in cholesterol transport by being the main protein component of high-density lipoprotein particles in the plasma. Over 50 ApoAI variants are known, of which more than 20 have been associated with hereditary ApoAI amyloidosis (Moutafi et al., 2019). Probably the mutations increase the risk of proteolysis and result in generation of amyloid fibril-prone fragments (Westermarck, Fandrich & Westermarck, 2015). In ApoAI amyloidosis extensive visceral amyloid deposits affect the liver, spleen, and kidney, occasionally also the heart, nerves, larynx, and gastrointestinal tract (Rowczenio et al., 2011). The clinical spectrum of ApoAI amyloidosis is very heterogeneous since the organ distribution, age of onset, clinical features, progression rate, and prognosis are dependent on the mutation site (Eriksson et al., 2009). Patients with N-terminal mutations mainly suffer from hepatic and renal amyloidosis, while patients carrying C-terminal mutations usually develop cardiac, laryngeal, and cutaneous amyloidosis (Eriksson et al., 2009). Overall, typical symptoms in ApoAI amyloidosis include hypertension, proteinuria and renal impairment (Rowczenio et al., 2011).

Lysozyme amyloidosis is a rare autosomal dominant hereditary amyloidosis caused by lysozyme derived amyloid deposits (ALys). Lysozyme is a ubiquitous bacteriolytic enzyme synthesized by hepatocytes, polymorphonuclear leucocytes and macrophages and so far eight different variants of lysozyme leading to amyloidosis have been reported (Li, Z. et al., 2019). Lysozyme amyloidosis mainly involves the digestive tract, liver, spleen, kidneys, lymph nodes, skin, and lachrymal and salivary glands. Also vascular involvement has been reported in many organs (Benyamine et al., 2017).

Other systemic amyloidosis with amyloid angiopathy are transthyretin amyloidosis (ATTR) and HGA.

### *Localised amyloid angiopathies*

Three different localised amyloidoses affect the vascular system. These are AANF, AMed and A $\beta$  related amyloidoses. All of them are age-related amyloidoses.

AANF related amyloidosis is also known as isolated atrial amyloidosis (IAA). The fibril protein deposited in IAA is the atrial natriuretic factor (ANF), a peptide hormone synthesized and secreted predominantly by atrial cardiomyocytes (Johansson, Wernstedt & Westermark, 1987). IAA is strictly localized to the atrium of the heart, mostly to the auricles, and very small vessels are commonly involved (Cornwell, Westermark, 1980). The incidence of IAA increases with age, reaching 90% in the ninth decade (Kawamura et al., 1995). Hypertension, diabetes mellitus, hypertrophy of the heart, coronary atherosclerosis, and dilatation of the atria show no significant relationship to the incidence or severity of IAA (Steiner, Hajkova, 2006).

AMed or medin amyloidosis (formerly senile aortic amyloidosis) is the most prevalent of amyloidoses. Amyloid is found in the *tunica media* of aortic walls of almost 100 % of the population above 50 years of age (Mucchiano, Cornwell & Westermark, 1992). The precursor protein for medin is lactadherin which is synthesized in VSMCs. Medin peptides are deposited locally to the aorta (Haggqvist et al., 1999) and may increase the risk for aortic rupture by killing the smooth muscle cells and inducing degradation of elastin and collagen (Peng et al., 2007).

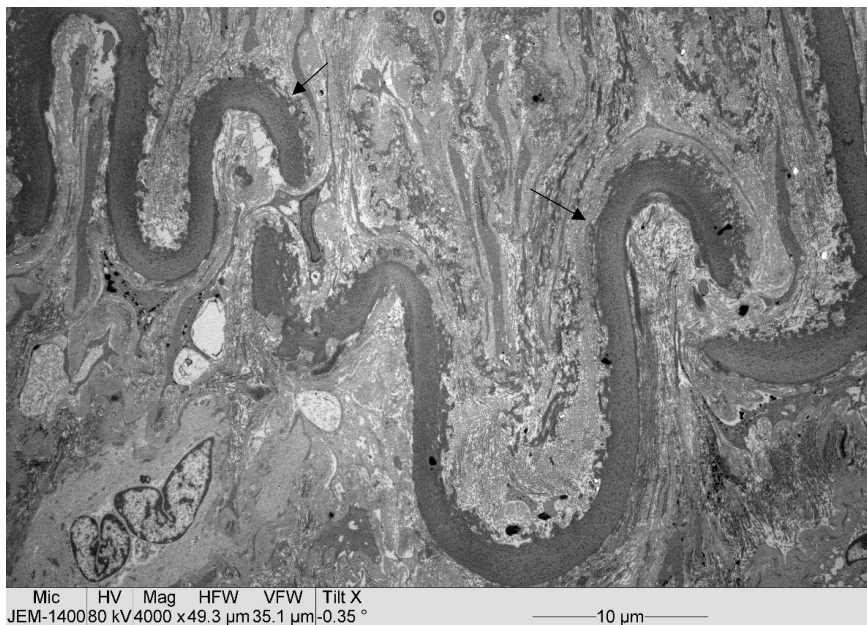
Alzheimer's disease is also a localised amyloidosis.

## **1.4 Elastic fibres**

Elastic fibres are essential insoluble components of the extracellular matrix (ECM) in dynamic tissues like skin, lungs, and blood vessels as they enable critical properties of elasticity and resilience. Elastic fibres are assembled in a highly organized process during development in mid-gestation and they are able to maintain their function for a lifetime (Kielty, Sherratt & Shuttleworth, 2002). Since elastic fibres play a vital role for example in the cardio-respiratory system, it has been suggested that age-related failures of elastic fibres could even be the limiting factor for a human life expectancy to 100-120 years (Robert, Robert & Fulop, 2008).

Elastic fibres are formed of an amorphous, crosslinked elastin core and a fibrillin-rich microfibrillar outer layer (Kielty, Sherratt & Shuttleworth, 2002) (Figure 3). Fibrillins are the

principal structural components of microfibrils. There are three different isoforms of fibrillin, fibrillin-1, -2 and -3, of which fibrillin-2 is expressed during early development and fibrillin-1 is predominant in mature microfibrils (Sherratt, 2009). Microfibrils form loosely packed parallel bundles in tissues (Kielty, Sherratt & Shuttleworth, 2002). Elastin is secreted as a soluble precursor protein, tropoelastin, and after a transfer to the microfibril scaffold it is enzymatically converted to a mature form by lysyl oxidase (LOX) or LOX-like proteins (Sherratt, 2009). Elastin is the most prevalent protein in elastic fibres comprising around 90 % of the structure (Baldwin et al., 2013). Elastin is a quite exceptional protein since it is extremely stable, resilient and also relatively resistant to proteinases, including elastases (Baud et al., 2013).



**Figure 3** Tortuous shaped lamina elastica interna (arrows) in artery of normal healthy individual seen in TEM analysis. Small dark dots are electron-dense microfibrils and lighter amorphous material is elastin.

Interestingly, elastin has a special role in AMed related amyloidosis since it is proposed to initiate amyloidogenesis by binding to lactadherin, which is the precursor protein of median amyloid (Larsson et al., 2006). In addition, elastin-like peptides interacting with heparan sulfate may directly generate amyloid (Boraldi et al., 2018).

### 1.4.1 Elastolysis

The degradation of mature elastin normally progresses very slowly, over years or decades. The rate and nature of age-related elastic fibre degradation varies between individuals and anatomical sites (Sherratt, 2009). This degradation process is called elastolysis and it is assumed to be caused by a disturbance in the normal balance between proteinases and their inhibitors (Werb et al., 1982). This can be observed, for example, in vessel walls during ageing and atherosclerosis (Kunecki, Nawrocka, 2001). Elastin can be degraded by aggressive proteolytic enzymes known as elastases (Baud et al., 2013). Human elastases have been identified within three different classes of proteinases: cysteine, serine, and metalloproteinases (Hornebeck, Emonard, 2011). Cysteine proteinases, mostly different types of cathepsins, are localized in lysosomes involved in intra-cellular degradation (Budd et al., 2013). Serine proteinases are a wide group of proteinases degrading components of the ECM. Serine elastases, which belong to this proteinases family, can be further divided into three different groups: pancreatic and neutrophil elastases, and cathepsin G. The first is secreted in the pancreas and has a major role in digestion, while the latter two are secreted in azurophil granules of neutrophils in inflammation, hence they are able to eliminate pathogens and break down tissues at inflammatory sites (Korkmaz et al., 2010).

The key players of ECM degradation in normal physiological processes are metalloproteinases, mainly matrix metalloproteinases (MMPs) (Budd et al., 2013). Matrix metalloproteinases contain  $Zn^{2+}$  ions/atoms and cleave the peptide bonds on the amino-terminal side. MMPs can be grouped into different subtypes according to their substrate specificity or cellular localization e.g. collagenases, gelatinases, stromelysins, matrilysins, metalloelastases and membrane type-MMPs. Four MMPs are known to be capable to degrade insoluble elastic fibres: MMP-2 (72 kDa gelatinase), MMP-7 (matrilysin), MMP-9 (92 kDa gelatinase) and MMP-12 (macrophage metalloelastase) (Mecham et al., 1997). The cascades between proteinases are complex since many proteinases regulate the activity of others by cleaving the protein precursors (pro-proteins) into mature active forms. Many MMPs have this characteristic, for example, pro-MMP2 is a substrate for MMP-14 (Budd et al., 2013).

Elastases can efficiently bind elastin but also onto cell surface-associated proteins such as heparan sulfate proteoglycans (Hornebeck, Emonard, 2011). Immobilization of elastin at the cell surface creates a microenvironment that favours elastolysis. Elastin peptides generated in

elastolysis are, among other properties, potent inducers of protease expression amplifying elastin degradation (Hornebeck, Emonard, 2011) and can interestingly even form amyloid-like fibers (Bochicchio, Pepe & Tamburro, 2007). Also, reactive oxygen species, calcification, aspartic acid racemization, lipid accumulation and mechanical fatigue may cause alteration of the elastic fibre structure favouring elastolysis (Robert, Robert & Fulop, 2008, Sherratt, 2009).

Elastases are inhibited by several different enzymes, for example, serine proteases are inhibited by serpins such as plasminogen activator inhibitors (PAI) and MMPs are inhibited by tissue inhibitors of metalloproteinases (TIMPs) (Budd et al., 2013). Additionally, many other factors also regulate the activity of proteinases. As an example, transforming growth factor- $\beta$  (TGF $\beta$ ) reduces collagenase production and stimulates the expression of TIMPs, leading to an overall inhibition of ECM degradation and resulting in excessive ECM accumulation (Akhurst, Hata, 2012). On the other hand, MMP-2 and MMP-9 cleave latent TGF $\beta$  in to an active form (Akhurst, Hata, 2012).

#### **1.4.2 Amyloid deposition on elastic fibres**

Amyloid fibrils tend to accumulate along elastic fibers (Winkelmann, Peters & Venencie, 1985, Sepp et al., 1990, Mucchiano, Cornwell & Westermark, 1992). The microenvironment on the surface of elastic fibre must be somehow favourable for the amyloid deposition. It has been suggested that serum amyloid P component (SAP) has an important role in this. SAP is associated normally with microfibrils of elastic fibers in healthy individuals (Breathnach et al., 1983, Breathnach, Pepys & Hintner, 1989). Unfortunately it also binds effectively to different types of amyloid fibrils that could encourage amyloid accumulation on the elastic fibre (Pepys et al., 1979, Pepys et al., 1982). AGel deposits have been reported to include SAP as well (Kiuru, Salonen & Haltia, 1999). SAP may also form a nidus for amyloid deposition (Winkelmann, Peters & Venencie, 1985). On the other hand, SAP is shown to act as an elastase inhibitor protecting not only normal elastic fibers but also pathological amyloid fibrils from proteolytic cleavages (Li, J. J., McAdam, 1984, Sepp et al., 1990).

#### **1.4.3 Elastolysis in amyloidoses**

Although amyloid fibril accumulation along elastic fibres is a widely recognized phenomenon, the fragmentation of elastic fibres, elastolysis or elastosis, is rarely reported. In other

conditions, like in atherosclerosis and UV-induced photoaging elastolysis, fragmentation of elastic fibres has been under intensive research, whereas only six cases are reported of amyloid elastosis to date. All of these cases are considered cutaneous amyloidosis, both systemic and localized amyloidosis types (Winkelman, Peters & Venencie, 1985, Sepp et al., 1990, Vecchietti et al., 2003, Bocquier et al., 2008, Santos-Briz et al., 2010, Marchand et al., 2013). Also, in HGA elastolysis has been described in the skin (Kiuru-Enari, Keski-Oja & Haltia, 2005). Apart from these, only a few cases of elastolysis (or elastosis) of other amyloid types have been reported so far, for example of CAA (Tian, Shi & Mann, 2004).



## **2 Hereditary gelsolin amyloidosis**

Hereditary gelsolin amyloidosis (HGA) is a rare systemic amyloidosis inherited in an autosomal dominant pattern (Meretoja, 1969, Kiuru-Enari, Haltia, 2013). HGA is also known as gelsolin-related amyloidosis (AGel amyloidosis), familial amyloidosis Finnish type (FAF), familial amyloid neuropathy type IV and Meretoja disease (OMIM #105120). It was originally reported in Finland by an ophthalmologist Jouko Meretoja in 1969 who recognized this new disease and later diagnosed more than 250 patients in Finland (Meretoja, 1969, Meretoja, 1976). HGA is a member of the Finnish disease heritage which includes 36 different inherited diseases that are more frequent in Finland than in any other population. Although HGA is nowadays reported worldwide, Finland still has the largest known patient population with an estimated prevalence of 1:6 000 (Norio, 2003) or even 1:1 040 in Kymenlaakso area (Meretoja, 1973). With the prevalence this high HGA is considered one of the most common diseases of the Finnish disease heritage. According to the latest haplotype analysis of Finnish HGA families, all Finnish HGA patients have the same ancestor (Mustonen et al., 2018), as Meretoja proposed already some decades ago (Meretoja, 1976). HGA can appear in heterozygous or homozygous forms.

Despite the patients of Finnish origin, the distribution of HGA reaches all over the world. At present, HGA has been identified already in many European countries, the United States, Japan, Brazil, Iran (Kiuru, 1998, Kiuru-Enari, Haltia, 2013), India (Maramattom, Chickabasaviah, 2013), Mexico (Gonzalez-Rodriguez et al., 2014), Canada (Alabdali et al., 2015), Korea (Park et al., 2016), and Argentina (Lucero Saa et al., 2017). Although HGA could still be underdiagnosed (Ardalan, Shoja & Kiuru-Enari, 2007) or even misdiagnosed (Juusela, P. et al., 2009), the growing awareness of HGA enables new cases to be found continuously from different parts of the world.

### **2.1 Gelsolin**

Gelsolin was found in 1979 by Yin and Stossel who isolated an unknown protein from rabbit lung macrophages (Yin, Stossel, 1979). They noticed that this protein binds to actin and modulates the network structure of actin filaments by shortening them. In the cells cytoplasmic actin can be at more firm state as a gel (gel) or at more soluble state (sol) and it

is continuously treadmilling in this gel-sol transformation. Because the unknown protein was found to be a regulator of this process it was named gelsolin. Actually gelsolin was also found by two other research groups at about the same time but they called this protein as actin-destabilizing factor (Chaponnier et al., 1979) and brevin (Harris, D. A., Schwartz, 1981, Wilkins, Schwartz & Harris, 1983), respectively.

### *Different isoforms*

Gelsolin has different isoforms, a cytosolic and a secreted isoform. The cytosolic isoform was the first one to be described (Yin, Albrecht & Fattoum, 1981) but soon also the secreted isoform was found from plasma (Yin et al., 1984). These two isoforms are generated by an alternative transcriptional initiation site and mRNA splicing from the single gelsolin *GSN* gene on chromosome 9 (9q33.2) (Kwiatkowski et al., 1986, Kwiatkowski, Westbrook et al., 1988). The cytosolic gelsolin contains 731 amino acids and its molecular mass is 81 kDa whereas secreted gelsolin contains 782 amino acids and its molecular mass is 86 kDa (UniProt - P06396 GELS\_HUMAN). The secreted gelsolin has a 24 amino acid long extension and a 27 amino acids long signal sequence in the N-terminus. It also has a disulfide bond between cysteine residues at positions 188 and 201 (Wen et al., 1996), and is more positively charged than the cytosolic isoform (Yin et al., 1984). The 24 amino acids N-terminal extension might have some functionality since it takes a defined, fixed position on the surface of the secreted gelsolin (Fock et al., 2005).

Furthermore, a third isoform, gelsolin-3, has been described. Gelsolin-3 is a cytosolic protein which has 11 additional residues at the N-terminus. Gelsolin-3 is expressed mainly in the brain, lungs and testis, but its specific function is still unknown (Vouyiouklis D.A., Brophy P.J., 1997).

### *Actin binding and modulation*

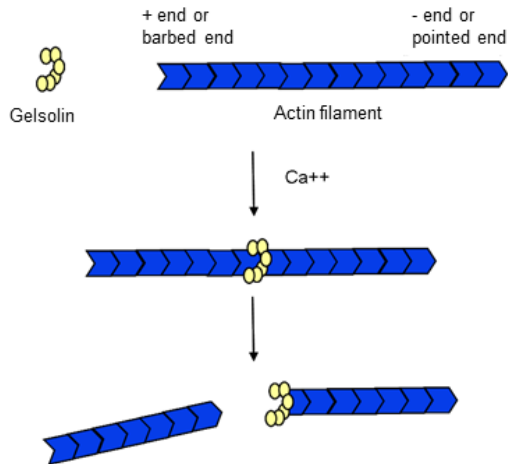
Actin is a highly conserved protein (molecular mass 42 kDa) forming microfilaments in the cytoplasm. Actin is the most abundant protein in most eukaryotic cells and it has six isoforms encoded by six different genes:  $\alpha$ -skeletal,  $\alpha$ -cardiac,  $\alpha$ -smooth,  $\gamma$ -smooth,  $\beta$ -cytoplasmic and  $\gamma$ -cytoplasmic isoforms (Perrin, Ervasti, 2010). Actin occurs in cells both as a monomeric globular protein (G-actin) and polymerized into actin filaments (F-actin). The actin cytoskeleton, formed of microfilaments in the cells, is responsible for many of the structural

and viscoelastic properties of the cells and critical for the cell motility (Dominguez, Holmes, 2011).

Actin participates in more protein-protein interactions than any known protein. About 300 actin-binding proteins (ABPs) are reported in total and more than 100 of those have established roles in modulation of actin filaments (Nag et al., 2013). Gelsolin is a founding member of one superfamily of actin-binding proteins. Gelsolin superfamily includes at least six other proteins in addition to gelsolin: villin, adseverin (also known as scinderin), capG, advillin, supervillin and flightless I (Kwiatkowski, 1999, Silacci et al., 2004, Nag et al., 2013). Of those, adseverin has the highest degree of homology (60 %) with gelsolin (Lueck, Brown & Kwiatkowski, 1998).

Gelsolin contains six gelsolin-like domains named G1 to G6 from the N- to the C-terminus. Each one of the domains comprise 97-118 residues folded into a 5- or 6-stranded  $\beta$ -sheet between a long helix that is approximately parallel, and a short helix that is approximately perpendicular, to the strands in the sheet (Burtnick et al., 1997, Burtnick, Robinson & Choe, 2001). Gelsolin is divided into two homologous halves, both containing threefold repeats: G1–G3 and G4–G6 (Kwiatkowski et al., 1986). Domains G1 and G4 contain the G-actin-binding sites whereas G2 binds to F-actin. In calcium ion ( $\text{Ca}^{2+}$ ) free environment G1-G6 domains pack together to a compact globular structure where actin binding sequences are inside of this structure and cannot interact with actin (Burtnick, Robinson & Choe, 2001).

Gelsolin severs actin filaments stoichiometrically and forms a cap structure to the barbed (+) -end of the newly generated actin filament which prevents reannealing of actin fragments (Harris, H. E., Weeds, 1984, Sun et al., 1994). Severing proceeds at the pointed (-) -ends, resulting in the rapid disassembly until an equilibrium is established between capped filaments and free gelsolin (Figure 4). Actin filament severing requires domains G1 and G2 (Kwiatkowski, Janmey & Yin, 1989, Way et al., 1989).



**Figure 4** Actin filament severing by gelsolin. Gelsolin severs actin filaments at the pointed (-) -ends and forms cap structures to the barbed (+) -end of the newly generated actin filament which prevents reannealing of actin fragments.

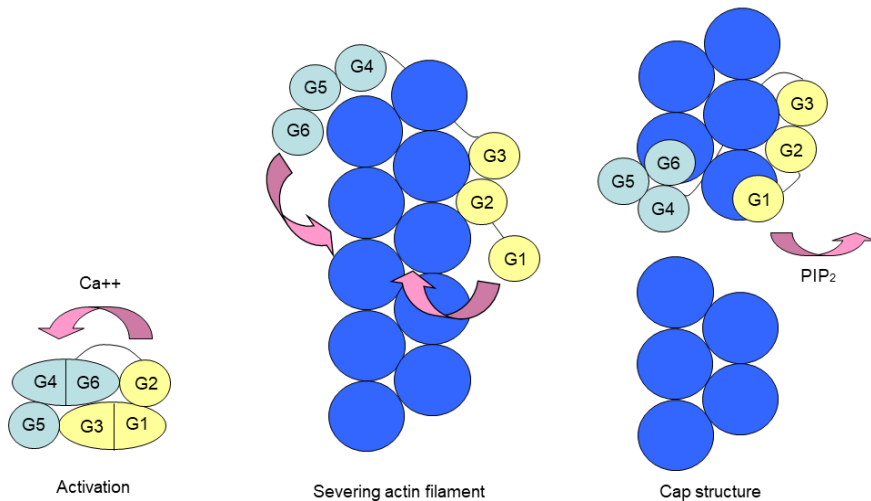
### Regulation

Gelsolin activity is regulated by  $\text{Ca}^{2+}$  (Yin, Zaner & Stossel, 1980, Yin, Albrecht & Fattoum, 1981, Yin et al., 1981, Janmey et al., 1985), intracellular pH (Selve, Wegner, 1987), phosphoinositides (Janmey, Stossel, 1987, Lin et al., 1997) and tyrosine kinase phosphorylation of gelsolin (De Corte, Gettemans & Vandekerckhove, 1997).

The isolated N-terminal half can bind and cap two actin monomers and can sever F-actin without free  $\text{Ca}^{2+}$  whereas the C-terminal half binds a single actin only when free  $\text{Ca}^{2+}$  is present.  $\text{Ca}^{2+}$  dependent regulation is based on C-terminal tail helix latch mechanism where the tail helix is responsible for transmitting  $\text{Ca}^{2+}$  binding from the C-terminal half to the N-terminal half of gelsolin (Lueck et al., 2000). When  $\text{Ca}^{2+}$  in micromolar concentration unlatches the tail helix, the F-actin binding site on G2 will become exposed and the G3–G4 linker adopts an extended conformation that enables the two halves of gelsolin to separate from each other. Subsequent opening of the G1–G3 and G4–G6 allows remarkable rearrangement of the relative positions of the domains which is required for active conformation of gelsolin (Lin, Mejillano & Yin, 2000, Choe et al., 2002) (Figure 5). The tail helix latch is responsible also for the unusual temperature sensitivity of gelsolin (Lueck et al., 2000).

Intracellular pH is another essential factor regulating actin cytoskeleton reorganization via gelsolin. It has been shown that severing activity decreases with pH deviating from neutral. This is result from a structural and/or charge changes of gelsolin and actin molecules (Selve, Wegner, 1987, Lamb et al., 1993). At lower pH gelsolin does not need  $\text{Ca}^{2+}$  and at higher pH gelsolin and actin are departed because of the repulsion force.

Phosphatidylinositol 4,5-bisphosphate ( $\text{PIP}_2$ ) is a regulator of gelsolin that favors filament uncapping and actin polymerization (Janmey, Stossel, 1987).  $\text{PIP}_2$  inhibits interactions between free gelsolin and actin. It also uncaps actin filaments by disrupting pre-existing interactions with gelsolin (Janmey, Stossel, 1987). Binding sites for  $\text{PIP}_2$  on gelsolin are within the G1-G2 linker (residues 135–142), in an area that overlaps the G2 F-actin-binding site (residues 161–169), and in the G5-G6 linker (residues 620–634) (Feng et al., 2001). The binding is modulated by calcium and pH (Lin et al., 1997).



**Figure 5** Actin filament severing by gelsolin. In the first phase gelsolin is activated by calcium ions ( $\text{Ca}^{2+}$ ) that opens the structure of gelsolin. In the second phase the N-terminal side of gelsolin binds to actin when G1 domain's active site is able to interact with actin leading to severing of actin filament. In the third and last phase C-terminal side of gelsolin binds to barbed end (+-end) of actin filament and forms cap structure.  $\text{PIP}_2$  is able to release gelsolin from this cap structure. Modified from Sun et al. (Sun et al., 1999).

### *Distribution and function*

Gelsolin is mainly produced by the smooth and striated muscle cells, although it is expressed in various cell and tissue types including nervous tissue (Kwiatkowski, Mehl & Yin, 1988, Kwiatkowski, Mehl et al., 1988, Tanaka, J., Sobue, 1994, Paunio, T. et al., 1997). In the cytoplasm gelsolin is located in the sites that are rich in actin filaments (Cooper et al., 1988). Properties of the cytoskeleton depend on parameters, such as actin filament length, flexibility, concentration and presence of cross-links. Cytosolic gelsolin participates the regulation of cellular morphology, function, and motility of cells by severing, capping and nucleating activities on actin filaments (Silacci et al., 2004).

The roles of secreted gelsolin are also associated to actin severing. Gelsolin is a crucial factor in apoptosis in different ways. In cellular death and lysis, actin is released into extracellular space ending up in blood plasma. Ionic strength, pH and temperature in the plasma favor the elongation of F-actin filaments which could interfere blood flow through in microcirculatory vessels (Burtnick, Robinson & Choe, 2001). Together with other plasma actin-binding protein, vitamin D-binding protein (DBP), gelsolin appears to "neutralize" actin in its monomer and polymeric forms (Haddad et al., 1990). In apoptosis gelsolin is cleaved from a long linker site connecting homologous halves (G1-G3 and G4-G6) by caspase-3 and after that it is able to cleave actin without  $\text{Ca}^{2+}$  regulation (Sun et al., 1999). Excess of N-terminal gelsolin induces apoptosis, whereas gelsolin null neutrophils have delayed onset of apoptosis (Kothakota et al., 1997). On the other hand, gelsolin has suggested to act also as an inhibitor of the apoptotic process (Silacci et al., 2004). For example, Jurkat cells exhibited a phenotype more resistant to apoptosis when gelsolin was expressed threefold more than in wild-type cells (Ohtsu et al., 1997). The expression of gelsolin is specifically down-regulated in 60-90 % of tumours during carcinogenesis of breast, colon, stomach, bladder, prostate, and lung (Tanaka, M. et al., 1995, Asch et al., 1996, Kuzumaki et al., 1997, Spinardi, Witke, 2007). These observations suggest that gelsolin down-regulation in tumours may be one mechanism for tumours to evade apoptosis. Interestingly, the possible protective effect of gelsolin in cancer could be evident in HGA patients as they have higher concentration of gelsolin in plasma (Paunio, Tiina et al., 1994) and less fatal cancers compared to general Finnish population (Schmidt et al., 2016).

Gelsolin has an important role in the cellular response of inflammation. In addition to actin severing, gelsolin is known to participate in Fc-receptor and integrin-mediated phagocytosis, but not in complement-mediated phagocytosis (Li, G. H. et al., 2012). In gelsolin null mice the

hemostatic, inflammatory, and fibroblast responses are blunted (Witke et al., 1995). Other studies have shown that the plasma gelsolin level decreases dramatically as a result of major trauma, and that reinfusion of recombinant gelsolin can protect against lung damage associated with major burn injury and other types of insults (Silacci et al., 2004). Low gelsolin level (less than 25 % of normal level) might predict secondary inflammation and tissue injury and that can be an indicator of critical care complications (Bucki et al., 2008). For this same reason gelsolin has been suggested to be used as a prognostic marker in sepsis (Horvath-Szalai et al., 2017). Gelsolin is also involved in chronic inflammatory diseases such as rheumatoid arthritis. It has been confirmed that gelsolin expression is lost in rheumatoid synovial fibroblasts leading to severe alterations in cytoskeletal organization (Aidinis et al., 2005).

Interestingly, gelsolin might have a role in Alzheimer's disease as well. Gelsolin has been shown to behave as an anti-amyloidogenic, anti-oxidant and anti-apoptotic factor in a transgenic mouse model of Alzheimer's disease where gelsolin forms a complex with A $\beta$  protein and reduces the amyloid load (Chauhan, Ji & Chauhan, 2008). It has also been shown that gelsolin is proteolytically cleaved by caspase-3 in the brains of individuals with Alzheimer's disease probably due to the oxidative stress (Ji et al., 2009).

Gelsolin is related to different types of cardiac injuries, including pressure overload, dilated and ischemic cardiomyopathy, myocardial infarction, and end-stage heart failure where the expression of gelsolin is increased (Li, G. H. et al., 2012). Gelsolin is present also in human platelets (Lind, Yin & Stossel, 1982). Platelet activation by either G- or F-actin may be regulated by the local concentrations of gelsolin and DBP. These two proteins inhibit platelet aggregation in a manner that can be explained by their effects on actin's filament structure (Vasconcellos, Lind, 1993).

Other functions of gelsolin are related for example to multiple sclerosis where gelsolin decreases actin toxicity and inflammation in a mouse model (Li-ChunHsieh et al., 2015). In airway smooth muscle relaxation and activation of gelsolin may contribute to relaxation (Mikami et al., 2017). Gelsolin has also been isolated in complexes of integrins, lipases and kinases. Gelsolin may alter lipid signaling pathways, either through direct binding to these proteins or through joint binding to clustered phosphoinositides (Kwiatkowski, 1999). Furthermore, gelsolin regulates Rac expression and is a downstream effector of the Ras-PI3K signaling pathway in gelsolin-induced cellular invasion. In phagocytosis of collagen, gelsolin associates functionally with  $\beta$ 1 integrins and Rac (Li, G. H. et al., 2012).

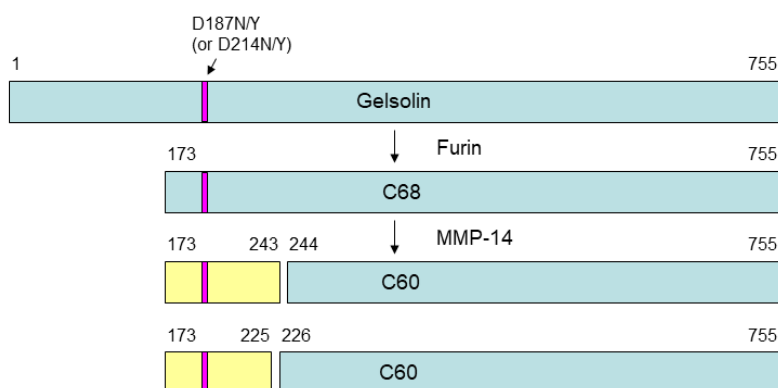
### 2.1.1 Causative mutation of gelsolin

Originally systemic HGA was found to be caused by two different mutations of the same codon in the *GSN* gene, c.640G>A or c.640G>T (formerly G654A or G654T) (Levy et al., 1990, Maury et al., 1990). On the protein level these mutations cause substitutions p.D187N or p.D187Y (also known as p.D214N or p.D214Y when the nomenclature includes the 27-residue signal peptide) (Ghiso, J. et al., 1990, Haltia et al., 1990, Maury, 1991, de la Chapelle et al., 1992). The mutated, or variant, gelsolin protein is misfolded, therefore it undergoes abnormal enzymatic cleavages by furin and matrix metalloproteinase 14 (MMP-14). First cleavage takes place in the Golgi complex by furin (Chen et al., 2001, Huff et al., 2003) and it generates a C-terminal fragment termed C68 (Maury, Rossi, 1993, Paunio, T. et al., 1994). The second cleavage occurs on the cellular plasma membrane by MMP-14 (Page et al., 2005). As an end result of these pathological cleavages the gelsolin protein turns to amyloidogenic fragments, sized 5 and 8 kDa (Figure 6). Further these fragments form AGel fibrils at extracellular sites of many tissues (Solomon et al., 2009). Fibril formation is accelerated by heparin (Suk et al., 2006, Solomon et al., 2011), 1-palmitoyl-2-(9'-oxononanoyl)-sn-glycero-3-phosphocholine (Mahalka, Maury & Kinnunen, 2011), and apolipoprotein E (Soto et al., 1995). The amyloidogenicity of gelsolin is also controlled by pH (Ratnaswamy et al., 1999) and  $\text{Ca}^{2+}$  binding (Page et al., 2004).

The mutation locates in the G2 domain in gelsolin and affects both regulatory ( $\text{Ca}^{2+}$ ) and actin-binding sites in gelsolin (Sun et al., 1999, Burtnick et al., 2004, Nag et al., 2009). It is presented that pathological cleavage cascade of variant gelsolin is actually initiated by the inability of the mutant domain 2 to bind  $\text{Ca}^{2+}$  (Page et al., 2004). It is also shown that any disruption in G2 and G3 domain interaction leads to increased furin cleavage (Zorgati et al., 2019). However, only the secretory form of gelsolin is assumed to be the source of amyloid in HGA (Kangas et al., 1996). In plasma the actin severing and nucleating activity of variant gelsolin is less than 5 % of normal values in homozygous HGA patients and in heterozygous HGA patients the severing activity is reduced by about 50 % in plasma (Weeds et al., 1993).

Other mutations in the *GSN* gene causing AGel fibril accumulation have been found quite recently; c.580G>A (p.G167R) (Sethi et al., 2013, Boni et al., 2018), c.633C>A (p.N184K) (Efebera et al., 2014, Boni et al., 2016) causing renal amyloidosis, and c.1375C>G causing amyloidosis with atypical symptoms (Oregel et al., 2018).





**Figure 6** Proteolytic cleavage of the variant gelsolin. The structure of the variant gelsolin opens for the furin cleavage and then the C68 fragment is cleaved further to C60 and amyloidogenic fragments (5-8 kDa, yellow). The mutation site is shown in purple.

## 2.2 Pathology

AGel accumulates primarily at the basal lamina of various types of epithelial cells, smooth, skeletal and cardiac muscle cells, and in the epi- and perineurium of peripheral nerves and in meninges (Meretoja, Teppo, 1971, Haltia et al., 1990, Kiuru et al., 1994, Kiuru et al., 1995, Kiuru-Enari et al., 2002). Deposition of AGel in arterial walls is encountered in nearly every organ including skin, muscle, rectum, nerve, kidney and salivary glands (Meretoja, Teppo, 1971, Kiuru, 1992, Kiuru et al., 1994, Kiuru-Enari et al., 2002, Juusela, P. et al., 2013, Pihlmaa et al., 2016). In the eyes AGel deposits are found especially in the corneal lattice lines, under Bowman's membrane, and in the scleral drainage area. It is suggested that AGel amyloid is produced locally in the eyes, especially in the cornea, conjunctiva, sclera, and ciliary muscle (Kivelä et al., 1994). The increasing amount of amyloid is associated with the severity of clinical features in HGA (Pihlmaa et al., 2016).

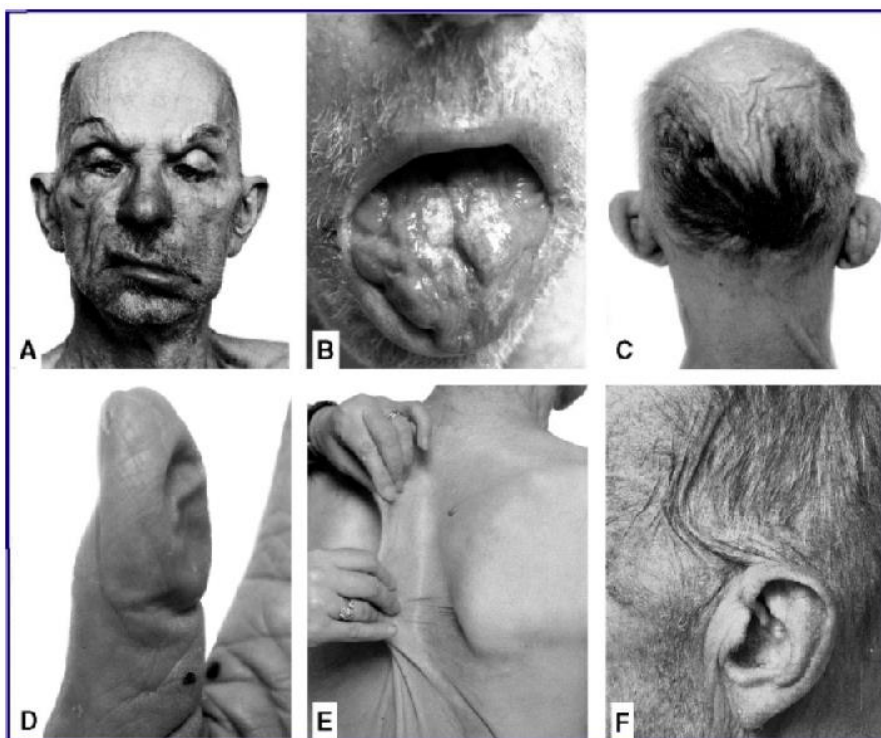
The pathomechanisms on cellular and molecular level causing HGA amyloidosis are not fully clarified. AGel depositions with subsequent cytotoxic effects are thought to contribute to the pathogenesis (Anan et al., 2010). Similarly, to other amyloidoses, the formation of oligomeric aggregates itself during the amyloidogenesis with proteotoxic and cytotoxic effects has been suggested to have pathogenetic significance in HGA (Solomon et al., 2012). Amyloid fibril

formation accelerators, specific pathological chaperons, have been recognised in AGel deposits as well, such as apo E, SAP and alpha-1-chymotrypsin (Kiuru, Salonen & Haltia, 1999).

## 2.3 Clinical features

In heterozygous patients HGA is a slowly progressing usually late-onset disease (Kiuru-Enari, Haltia, 2013) with no significant affect to the patients' life expectancy (Schmidt et al., 2016). However, it causes many difficulties to the patients already at a younger age (Laine et al., 2010). The main, and usually the first, symptom is corneal lattice dystrophy (CLD), that occurs at thirties or forties (Meretoja, 1969, Meretoja, 1973, Nikoskinen et al., 2015). This ophthalmological manifestation often causes recurrent corneal erosions and visual impairment or even loss of vision at an advanced age. Patients frequently suffer from dry and irritable eyes and photophobia (Meretoja, 1969, Kiuru, 1992).

Other characteristics of HGA are neurological and dermatological manifestations. Neuropathy involves most typically cranial and peripheral nerves, while autonomic and central nervous signs are mainly minor (Kiuru et al., 1994, Kiuru, Seppalainen, 1994, Kiuru et al., 1995). Patients usually develop distal paresthesias and manual clumsiness and slowly progressing, commonly bilateral facial nerve paresis (Pihlmaa et al., 2011). Dysphagia and dysarthria, balance problems, even prominent ataxia with severely impaired mobility can occur too, particularly in aged patients (Tanskanen et al., 2009). Loose and hanging skin, known as *cutis laxa*, is a prominent feature of HGA contributing for example to progressive premature facial aging and an increased fragility of the skin (Kiuru et al., 2000, Kiuru-Enari, Keski-Oja & Haltia, 2005, Pihlmaa et al., 2011) (Figure 7). Atrophy of the skin is associated with symptoms like itching and dryness, increased vulnerability, and abnormal scarring. In addition, the hair of the body and scalp can be diminished or lost. Together this characteristic clinical triad of neurological, dermatological, and ophthalmological manifestations can cause among other things severe facial disfigurement, hypomimia, and loss of vision, which severely decrease quality of life (Laine et al., 2010).



**Figure 7** Clinical characteristics of HGA in 79-year-old male patient. HGA patients have severe looseness of the skin (*cutis laxa*) contributing to many difficulties such as hypomimia, dysarthria and drooling together with facial and bulbar paralysis. A) Characteristic facial features with drooping appearance. Regardless of several corrective plastic surgeries the patient shows serious hanging of the eyelids and mouth. B) Tongue is furrowed and macroglossic. C-E) *Cutis laxa* affected abnormally lax, folded, and unelastic skin of the scalp, the thumb and the back. D and F) Lost elasticity is noted in a pressure test where the skin maintains the deformed state 2-5 min after pressure. Figures published with the permission of the British Journal of Dermatology (Kiuru-Enari, Keski-Oja & Haltia, 2005).

Apart from progressive facial nerve and cutaneous tissue impairment this amyloidosis also causes various oral symptoms. HGA patients have often reduced saliva secretion with problems of dry mouth, also periodontitis and, loose and cracked teeth (Juusela, P. et al., 2013, Juusela, P. L. et al., 2015). Furrowing and fasciculations of the tongue is typical in older patients, reminiscent of bulbar amyotrophic lateral sclerosis which may result even in misdiagnosis, while macroglossia is rare (Kiuru, Salonen & Haltia, 1999, Kiuru-Enari, Haltia, 2013). Patients suffer from socioaesthetic problems because of dysarthria, dysphagia and

drooling. Sleep apnea due to pharyngeal tissue laxity can also occur in HGA (Kiuru, Nieminen & Partinen, 1999).

Although the penetrance of HGA is 100 %, the clinical phenotype can vary greatly between different patients (Kiuru, 1998). There might be gender related issues as well since in women the symptoms and signs develop at younger age and are more common than in men (Atula et al., 2016). The homozygous form of HGA is very rare and has been reported only in few cases (Meretoja, 1973, Maury et al., 1992, Ardalan, Shoja & Kiuru-Enari, 2007). The homozygous patients have an early onset and potentially fatal outcome even before the age of 30 due to accumulation of amyloid in the kidneys. Renal involvement in HGA is characterized by mainly amyloid glomerulopathy, in severe cases with subsequent nephrotic syndrome (Maury, 1993). In heterozygotes amyloid glomerulopathy is usually subclinical, or associated with only minor proteinuria (Kiuru 1992). However, they may also manifest with severe renal involvement (Kiuru-Enari, Haltia, 2013, Yamanaka et al., 2013, Sethi et al., 2017), which based on recent studies seems to be more common than earlier considered (Nikoskinen et al., 2015).

### **2.3.1 AGel amyloid angiopathy**

In the vascular system AGel accumulation is mainly concentrated to small- and medium-sized arteries, arterioles and capillaries, while veins are only rarely affected (Meretoja, Teppo, 1971). In arteries, the *tunica media* particularly is encroached on by AGel deposits. HGA patients with *cutis laxa* have an increased fragility of the skin and risk for intracutaneous bleeding (Kiuru et al., 2000, Kiuru-Enari, Keski-Oja & Haltia, 2005). HGA patients express more hemostatic derangements, for example they often report superficial bruises after minor trauma, and may sometimes require blood transfusions after surgical operations (Kiuru et al., 2000, Laine et al., 2010). Although patients wounds seem to heal normally, they have a tendency for noticeable postoperative swelling and hematomas, which could relate to the amyloid angiopathy (Pihlmaa et al., 2011). HGA patients also suffer more often from cardiac diseases and arrhythmias, and the consumption of cardiovascular medication is significantly increased (Laine et al., 2010, Nikoskinen et al., 2015). Interestingly, a recent study shows that AGel deposits are present in the myocardium and cardiac blood vessels in all studied HGA patients (n=25) (Schmidt et al., 2019). Concerning nerve tissues, the AGel amyloid angiopathy is particularly prominent in proximal portions of the spinal nerves (Kiuru, Salonen & Haltia, 1999). It may explain the predominant large nerve fiber loss in HGA, which is not seen in other

amyloid neuropathies in general (Kiuru-Enari et al., 2002). Cerebral hemorrhages have also been reported. Signs of abnormal vascular permeability in cerebral tissues were observed in an HGA brain autopsy study (Kiuru, Salonen & Haltia, 1999). Minor neuropsychiatric changes in HGA could be involved with microhemorrhages or microcalcifications found in magnetic resonance imaging of brains in HGA patients (Kantanen et al., 2014).

## **2.4 Current treatment and future prospects**

Treatment at present is based only on alleviation of symptoms. Eyes are treated with lubricants and topical antibiotics when needed. Regular ophthalmological follow-up is important, apart from corneal and sicca problems patients can develop glaucoma and cataracts. Only in severe cases corneal transplantations are considered as the failure rate is about 50 % due to surface complications (Mattila et al., 2015). Facial symptoms are treated with plastic surgery but due to relentless disease progression patients need selected techniques and repeated surgery (Pihlmaa et al., 2011). A novel grading method for bilateral facial paralysis is established for evaluation of disease progression and necessity for the surgical treatment (Pihlmaa et al., 2015). Other skin problems, like itching and dryness, are relieved with ointments. Also, dryness in the mouth can be alleviate with suitable products but careful dental care is essential (Juusela, P. L. et al., 2015). Homozygous and rare heterozygous patients with severe nephrotic syndrome need dialysis or even kidney transplantation (Maury et al., 1992, Ardalan, Shoja & Kiuru-Enari, 2007, Nikoskinen et al., 2015). The outcome of renal transplant can be positive, without recurrence of proteinuria at least during a 6-year follow up (Shoja et al., 2009). For conduction abnormalities cardiological follow-up, sometimes pacemaker treatment, may be needed (Schmidt et al., 2019).

So far, specific treatment of HGA is unfortunately not available for patients. Hypothetically, on cellular level AGel formation could be prevented or diminished by inhibiting the enzymes in the cleavage cascade of variant gelsolin. It has indeed also been shown that for example with proprotein convertase inhibitors, like  $\alpha$ 1-antitrypsin Portland ( $\alpha$ 1-PDX), the furin cleavage of variant gelsolin can be inhibited (Kangas, Seidah & Paunio, 2002). However, the inhibition of furin and MMP-14 proteases would with very high probability encounter unwanted side effects, since both are involved in protein-trafficking pathways as well (Page et al., 2005).

A novel strategy for HGA therapy, which is inhibiting furin and MMP-14 without interfering other protein-trafficking pathways, is nanobodies which are protective molecular chaperones in the pathogenic cleavages of variant gelsolin (Van Overbeke et al., 2014, Van Overbeke et al., 2015). These nanobodies are further combined into a single bispecific format so they can simultaneously shield variant gelsolin from both proteolytic enzymes. This nanobody based approach is adeno-associated virus gene therapy and it has showed promising results in gelsolin amyloidosis mice (Verhelle et al., 2017).

There has been great success in the development of different therapies in TTR amyloidosis; the siRNA molecule patisiran (Onpattro) and antisense oligonucleotide inotersen (Tegsedi) were approved by FDA in 2018 (Gales, 2019) and the TTR tetramer stabilizer tafamidis (Vyndaqel and Vyndamax) in 2019 (Coelho et al., 2016). Unfortunately, this is not the case in other amyloidoses. Nevertheless, the same stabilizing strategy, as in tafamidis, could work also for AGel formation, if a pharmacologic chaperone or a more specialized kinetic stabilizer would be found to stabilize the structure of the G2 domain in variant gelsolin (Solomon et al., 2012).

SAP, a widely studied serum protein, plays an important role in clinical diagnostics when used in scintigraphy (Hawkins, Pepys, 1995) but it is also a possible drug target for anti-amyloid therapy (Pepys et al., 2002). Two drugs have been so far studied for this purpose; miridesap to deplete circulating SAP and a humanized monoclonal anti-SAP antibody dezamizumab to bind to residual SAP in amyloid deposits, produce unprecedented removal of amyloid from the tissues and improve organ function (Pepys, 2018). Miridesap was intended to target SAP associated with cerebral A $\beta$  amyloid deposits and cerebral vasculature but it could work also in systemic amyloidose, such as HGA (Pepys, 2018).

Interestingly, water soluble poly lactic-co-glycolic acid (PLGA)-encapsulated curcumin and emetine nanoparticles seem to be potential modulators of gelsolin amyloidogenesis (Srivastava et al., 2015). The curcumin nanoparticles augmented amyloid formation in gelsolin by skipping the pre-fibrillar assemblies and increased the fibrillar bulk, while the emetine nanoparticles induced non-fibrillar aggregates and were able to defibrillate the pre-formed AGel. Despite the functional differences in the aggregates induced by curcumin and emetine nanoparticles, both of them displayed reduced cellular toxicity (Srivastava et al., 2015).

## Aims of the study

The aims of this study were to characterise the pathological changes of AGel amyloid angiopathy in small arteries in more detail, to elucidate pathomechanisms of amyloid related elastolysis, and to investigate the effects of variant gelsolin in vascular smooth muscle cells from the HGA patients.

The specific aims can be outlined as follows:

- To make a thorough analysis of the pathological changes in the arterial wall in AGel amyloid angiopathy **(I)**.
- To characterise the elastolysis in AGel amyloid angiopathy and elucidate the pathomechanisms of the amyloid related elastolysis by analysing potential elastases from tissue and plasma samples **(II)**.
- To establish VSCM primary cell line cultures from the arteries of HGA patients and healthy controls and to study the biological effect of variant gelsolin **(III)**.

## Materials and methods

The methods used are summarized in a Table 2. The materials and methods are described in detail in the original publications as indicated.

Table 2.

Materials and methods	Original publication
<i>Transmission electron microscopy (TEM)</i> Stainings <ul style="list-style-type: none"> <li>➤ uranyl acetate and lead citrate</li> </ul> Examination <ul style="list-style-type: none"> <li>➤ JEM-1400 TEM with Morada TEM CDD camera</li> </ul>	I, II
<i>Histochemistry</i> <ul style="list-style-type: none"> <li>➤ hematoxylin eosin</li> <li>➤ Congo red</li> <li>➤ Herovici-elastica</li> </ul>	I
<i>Immunohistochemistry</i> Antibodies used against <ul style="list-style-type: none"> <li>➤ vAGel (rabbit polyconal)</li> <li>➤ <math>\alpha</math>-SMA (mouse monoclonal)</li> <li>➤ collagen type I (rabbit polyconal)</li> <li>➤ collagen type III (rabbit polyconal)</li> <li>➤ PINP (rabbit polyconal)</li> <li>➤ PIIINP (rabbit polyconal)</li> <li>➤ ICTP (rabbit polyconal)</li> <li>➤ TGF<math>\beta</math> (rabbit polyconal)</li> <li>➤ MMP-2 (mouse monoclonal)</li> <li>➤ MMP-7 (mouse monoclonal)</li> <li>➤ MMP-9 (mouse monoclonal)</li> <li>➤ MMP-12 (rabbit polyconal)</li> <li>➤ MMP-14 (rabbit polyconal)</li> <li>➤ TIMP-1 (mouse monoclonal)</li> </ul> Other reagents <ul style="list-style-type: none"> <li>➤ biotinylated secondary antibodies</li> <li>➤ ABC-reagent</li> <li>➤ diaminobenzidine (DAB)</li> <li>➤ fluorescent Cy5 secondary antibody</li> </ul> Examination <ul style="list-style-type: none"> <li>➤ Nikon Eclipse 800 microscope</li> <li>➤ Nikon Eclipse Ti-E inverted fluorescence microscope</li> </ul>	I, II
<i>Enzyme-linked immunosorbent assay (ELISA) analyses with commercial kits</i> <ul style="list-style-type: none"> <li>➤ MMP-2</li> <li>➤ MMP-7</li> <li>➤ MMP-9</li> <li>➤ MMP-12</li> <li>➤ MMP-14</li> <li>➤ TIMP-1</li> </ul>	II
<i>Generation of primary vascular smooth muscle cell lines</i> <ul style="list-style-type: none"> <li>➤ isolation and culturing of VSMC cells</li> </ul>	III
<i>Western blotting</i> Antibodies used against <ul style="list-style-type: none"> <li>➤ <math>\alpha</math>-SMA (mouse monoclonal)</li> </ul>	III



<p>Other reagents</p> <ul style="list-style-type: none"> <li>➤ Fluorescent IR 680 secondary antibody</li> </ul> <p>Examination</p> <ul style="list-style-type: none"> <li>➤ Odyssey Infrared Imaging System</li> </ul>	
<p><i>RNA isolation with commercial kits and sequencing</i></p> <ul style="list-style-type: none"> <li>➤ total RNA purification kit</li> <li>➤ cDNA synthesis kit for qRT-PCR</li> <li>➤ cycle sequencing kit</li> <li>➤ ABI 3130x1 capillary sequencer</li> </ul>	III
<p><i>Cyto- and immunocytochemistry</i></p> <p>Antibodies used against</p> <ul style="list-style-type: none"> <li>➤ gelsolin (mouse monoclonal)</li> <li>➤ <math>\alpha</math>-SMA (mouse monoclonal)</li> </ul> <p>Other reagents</p> <ul style="list-style-type: none"> <li>➤ phalloidin conjugated with fluorescent dye</li> <li>➤ fluorescent secondary antibodies</li> <li>➤ 4',6-diamidino-2-phenylindole (DAPI)</li> <li>➤ 3-amino-9-ethylcarbazole (AEC)</li> </ul> <p>Examination</p> <ul style="list-style-type: none"> <li>➤ Axioplan 2 fluorescence microscope</li> </ul>	III
<p><i>Cell migration analyses</i></p> <ul style="list-style-type: none"> <li>➤ colorimetric cell migration assay</li> <li>➤ scratch assay*</li> </ul>	III
<p><i>Chemical stimulation of the cells</i></p> <ul style="list-style-type: none"> <li>➤ phorbol 12-myristate 13-acetate (PMA)*</li> <li>➤ staurosporine treatment</li> </ul>	III
<p><i>Radioimmunoassay (RIA) with commercial kits</i></p> <ul style="list-style-type: none"> <li>➤ PINP</li> <li>➤ ICTP</li> </ul>	III
<p><i>Statistical analysis</i></p> <ul style="list-style-type: none"> <li>➤ Shapiro-Wilk test</li> <li>➤ student's t-test</li> <li>➤ Chi-square test</li> </ul>	II, III
<p><i>Image processing</i></p> <ul style="list-style-type: none"> <li>➤ ImageJ</li> </ul>	I, II, III
<p><i>Subjects</i></p> <ul style="list-style-type: none"> <li>➤ Samples included different types of material from <ul style="list-style-type: none"> <li>○ 35 patients with HGA (17 females and 18 males, mean age 60 years, range 35-86 years)</li> <li>○ 40 non-HGA-subjects (21 females and 19 males, mean age 53 years, range 18-78 years)</li> </ul> </li> </ul>	I, II, III

\* Nonpublished data.

# Results

## 1 AGel amyloid angiopathy

### 1.1 Characterization of the HGA patients

HGA patients of this study were diagnosed with HGA based on the typical clinical triad of lattice corneal dystrophy type 2, bilateral progressive facial paresis, and cutis laxa, along with family history (first degree relative/relatives with similar symptoms), and/or demonstration of the c.640G>A *GSN* mutation (32 of 35 HGA patients were genetically confirmed). Accumulation of amyloid in dermal and vascular tissue samples from HGA patients was verified by Congo red stainings and IHC with anti-vAGel antibody. HGA patients showed AGel deposition in arterial walls by red-green dichroism under polarized light microscopy in Congo red staining, and by immunopositivity for vAGel in IHC staining.

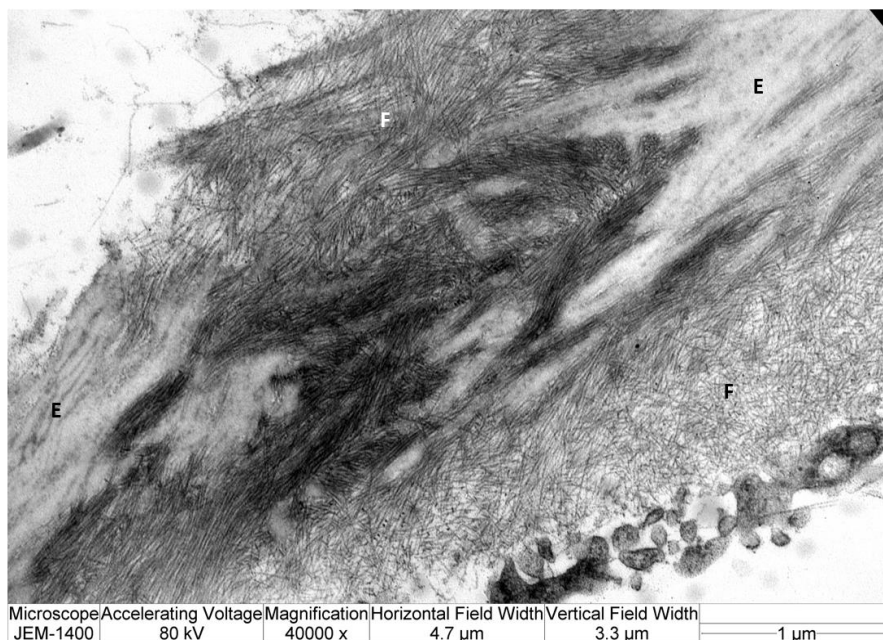
### 1.2 Structural changes in arterial walls

TEM showed clear degeneration and derangement of the arterial wall in the HGA patients. This was particularly evident in the *tunica media* and the *lamina elastica interna*. The vascular smooth muscle layer in the tunica media was disrupted. VSMCs were disorganized leaving large areas of extracellular matrix between the cells. Nevertheless, there were similar numbers of VSMCs in the patient and control arteries according to the results of anti- $\alpha$ -SMA staining.

### 1.3 Fibril accumulation

TEM analysis of both dermal and vascular tissue samples showed massive accumulation of fibrils along elastic fibres in all patients with HGA and in none of the controls (Figure 8). Also, amyloid fibrils, with a characteristic diameter of 8-10 nm, were detectable in all HGA patients throughout the *tunica media* as focal deposits. In addition to TEM, immunofluorescence demonstrated that AGel fibrils were clustered especially along the *lamina elastica interna*. In

TEM analysis were observed two types of fibrils: (a) the typical disordered non-branching amyloid fibrils and (b) long and parallel-oriented fibrils. The diameter (approximately 10 nm) was the same in all fibrils.



**Figure 8** TEM analysis of the arteries reveals accumulation of fibrils along cutaneous elastic fibre which is already severely damaged. (E) elastic fibre, (F) fibrils.

#### 1.4 Changes in collagen type I and III

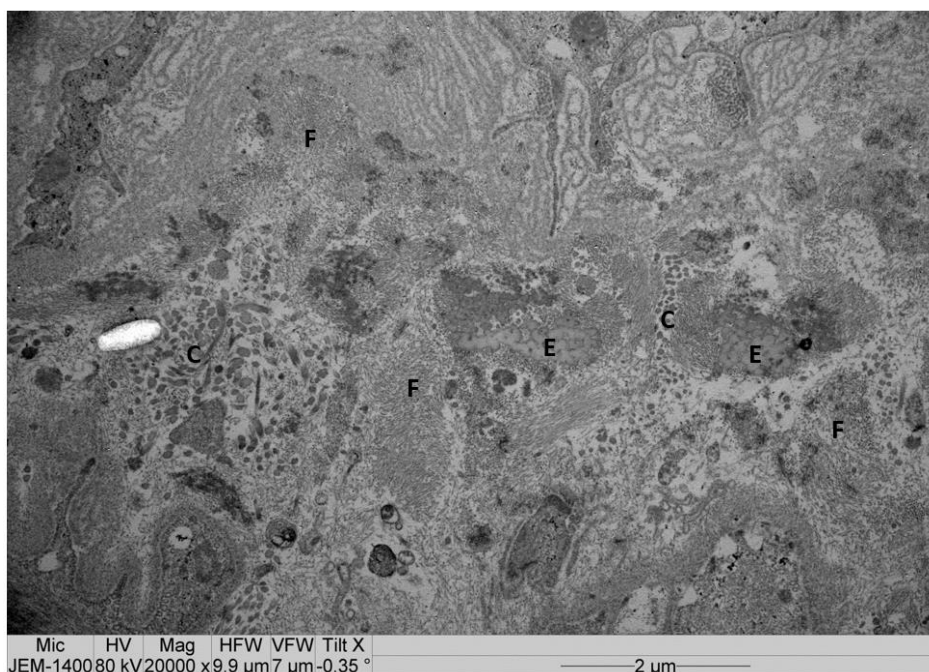
We studied the presence, synthesis and degradation of collagen types I and III in arteries as they are the most common collagen types in arterial walls (Shekhonin et al., 1985). In a semi-quantitative observation, the extent of immunopositivity for collagen types I and III was increased in HGA patients. Also, their localization in the *tunica media* was very different from that in the controls. Both collagen types were accumulated focally in HGA patients, compared to the control subjects, in whom collagens were distributed evenly forming netlike patterns. In anti-ICTP staining HGA patients' tissue samples showed more intense immunopositivity compared to the controls suggesting an increase of collagen type I degradation product. Immunopositivity for both ICTP and collagen type I were localized to the same abnormally

focally accumulated area. No differences between patient and control groups were noticed in the immunopositivity for PINP, PIIINP and IIINTP by semi-quantitative observation. Based on immunohistochemistry studies collagen type I and III synthesis and collagen type III degradation products were at the same level in both groups.

## **2 Elastolysis and potential pathomechanisms**

### **2.1 Fragmentation of elastic fibers**

In dermal and vascular tissue samples from HGA patients, the elastic fibers, especially the *lamina elastica interna* in arteries, were abnormally fragmented and diminished. This was discovered both in Herovici-elastica staining and in TEM (Figure 9). Occasionally, in the HGA patients the *lamina elastica interna* was entirely absent, indicating serious elastolysis and causing severe disorganization of the arterial wall. These phenomena were not observed in control subjects.



**Figure 9** TEM analysis of the arteries reveals massive accumulation of fibrils with a diameter of approximately 10 nm. Only small remains of the lamina elastica interna are left. (F) fibrils, (E) lamina elastica interna and (C) collagen.

## 2.2 Differences in the elastolysis pattern of elastic fibres

The degradation pattern of elastic fibres in dermal and vascular tissue samples was alike across all HGA patient samples, but differed remarkably from the less prominent age-related elastolysis seen in the control samples. Interestingly, it seemed that elastin of the elastic fibres was notably decreased or totally lost, whereas fibrils were still present forming a fibrous matrix. In the control subjects, the degradation appeared more granular, as in age-related elastolysis previously described by Braverman and Pasquali-Ronchetti (Braverman, Fonferko, 1982, Pasquali-Ronchetti, Baccarani-Contrì, 1997) and fibrils were not observed around the degraded elastic fibres.

## **2.3 Significance of MMPs**

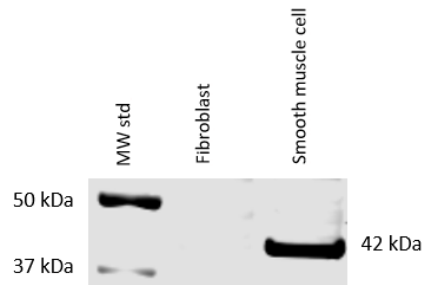
We measured the levels of MMP-2, -7, -9, -12 and -14 in the plasma samples. There was no difference in MMP-2, -7, and -12 levels between patient and control groups. In the plasma membrane-type MMP-14 could not be detected. However, MMP-9 levels were significantly lower in HGA patients vs controls: 0.22 ng/ml (SD 0.10) vs 0.57 ng/ml (SD 0.37),  $p = 0.002$ . These findings were further confirmed by analysing the IHC stained vascular tissue samples semi-quantitatively and quantitatively. In the MMP-9 immunostaining, a typical HGA patient had positive area ratio 2.15 times lower than control (mean intensity 2.17 times lower). No difference was seen in the IHC stainings for the other MMPs between the patient and control groups.

The previously reported cascade between MMP-9, TIMP-1 (inhibitor of MMP-9) and TGF $\beta$  (Akhurst, Hata, 2012, Kwak, 2013) led us to study the levels of TIMP-1 and TGF $\beta$  as this cascade may explain the decreased MMP-9 level in HGA patients. We analysed the vascular IHC samples semi-quantitatively and quantitatively and found distinct increase in the TGF $\beta$  immunoreactivity in a typical HGA patient vs control; positive area ratio 3.40 times higher in HGA patient than in control (mean intensity 3.35 times higher). Nevertheless, the level of TIMP-1 was identical in both studied groups in the plasma and tissue samples.

## **3 Biological effect of variant gelsolin in VSMCs**

### **3.1 Characterization of vascular smooth muscle cells**

VSMC lines were isolated from the same HGA patients as described in chapter 1.1. All cell lines were characterized to be VSMCs by their immunoreactivity to smooth muscle cell actin using  $\alpha$ -SMA-antibody in cell staining and Western blotting (Figure 10). Prevalence for positive staining was nearly 100 % for all VSMC lines, whereas fibroblasts showed negative staining with  $\alpha$ -SMA-antibody.



**Figure 10** Western blot analysis of fibroblast and vascular smooth muscle cell lines using anti- $\alpha$ -SMA-antibody. VSMCs show strong immunopositivity against  $\alpha$ -SMA whereas fibroblasts are totally negative.

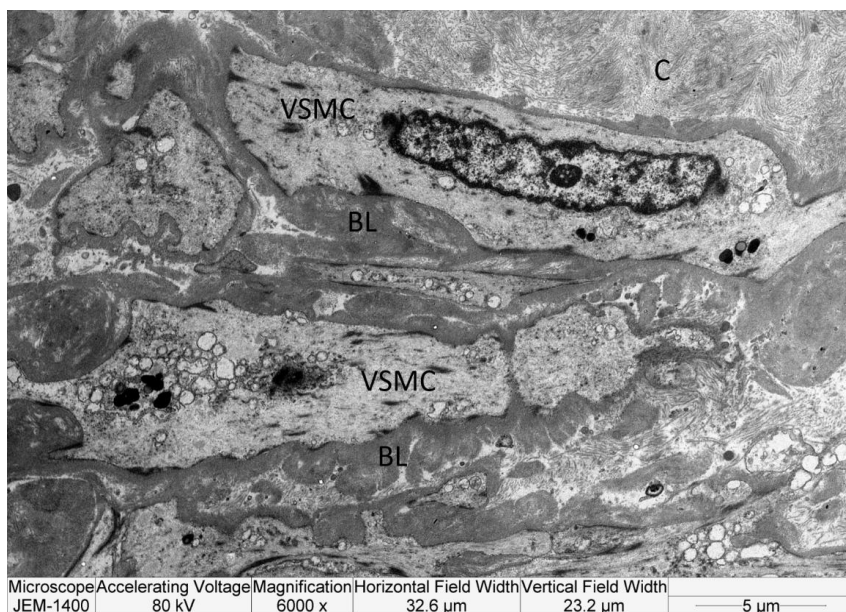
### 3.2 Expression of variant gelsolin

To confirm the expression of variant gelsolin we isolated the mRNA from the cells and sequenced the cDNA of three HGA patients (2 VSMC and 1 fibroblast cell lines) and one control (VSMC cell line). The sequences showed clearly that cell lines of HGA patients were expressing variant gelsolin in a ratio of approximately 1:1, whereas there was no sign of variant gelsolin in healthy controls. Yet, the C68 fragment of the gelsolin protein was not detectable in cell lysates or culture media of HGA patient cells. This is consistent with previous studies where only full-length gelsolin was found in the culture media of human fibroblasts transduced with recombinant adenoviruses encoding variant secretory gelsolin and also one HGA patient fibroblast cell line (Paunio, T. et al., 1998).

### 3.3 Morphology of VSMCs

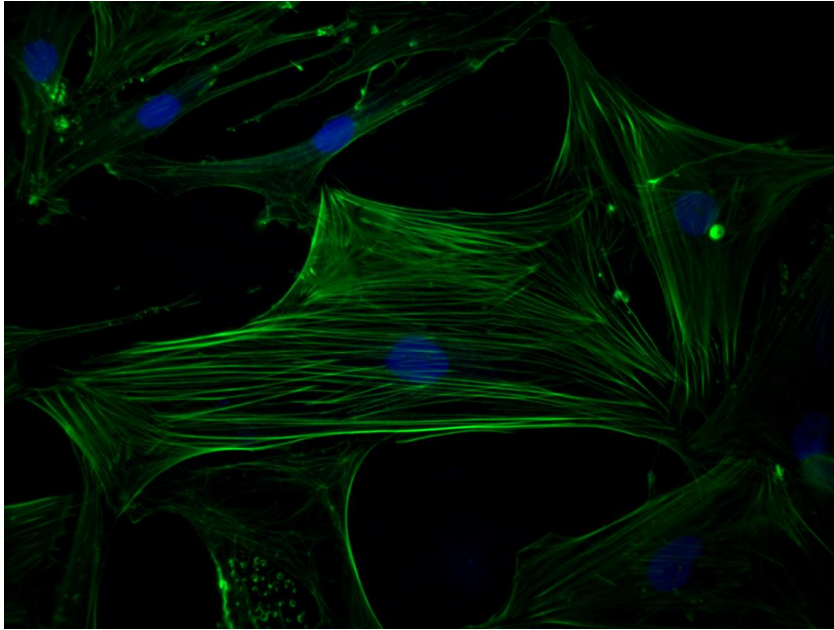
In the arteries the morphology of individual VSMCs showed otherwise normal in TEM analysis, but often the basal lamina of VSMCs was thickened (Figure 11). The number of VSMCs in arteries was also comparable between HGA patients and control subjects. In *in vitro* studies the general morphology of actin cytoskeleton and cytosolic gelsolin distribution were evaluated using fluorescent cell stainings (Figure 12). No significant differences in actin cytoskeleton morphology was evident, when comparing cells lines from HGA patients and

healthy controls. Both cell types, VSMCs and fibroblasts, grew in an elongated way and formed orientated parallel bundles of cells in *in vitro* culture.



**Figure 11** In TEM analysis of the arteries the morphology of individual VSMCs showed normal, only the basal lamina was often thickened and filled with AGel fibrils. (VSMC) vascular smooth muscle cell, (BL) basal lamina and (C) collagen.



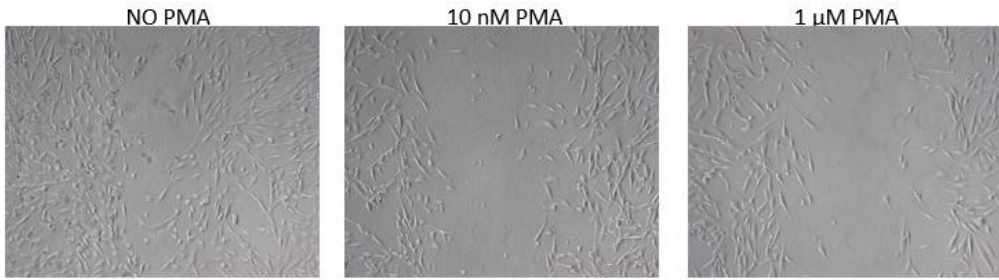


**Figure 12** Morphology of actin cytoskeleton was evaluated using fluorescent cell stainings: phalloidin for actin cytoskeleton (green) and DAPI for nucleus (blue). The VSMCs in the image are from HGA patient. Magnification x 400.

### 3.4 Cell migration, PMA and staurosporine treatments

Migration of the VSMCs was analysed with a commercial a colorimetric cell migration assay (Chemicon International, Billerica, MA) and with a scratch assay (Liang, Park & Guan, 2007). The cells showed no notable difference in the migration ability between HGA patients and healthy controls.

Phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, treatment was performed combined with a scratch migration assay (Figure 13). Cells were incubated 72 hours in different concentrations (0 M, 10 nM and 1  $\mu$ M) of PMA and data was collected at different time points (0, 24, 48 and 72 h). The cells in both studied groups behaved in similar manner.



**Figure 13** PMA treated cells were studied in a scratch migration assay. This image series shows an effect of PMA in different concentrations at time point 24 h in HGA patient VSMCs.

Staurosporine, a protein kinase C inhibitor, treatment was also performed. It has the effect of rapid disruption of actin microfilaments by inhibiting actin-cytosolic gelsolin induction (Miyamoto, Wu, 1990, Yu, Gotlieb, 1992). However, in VSMCs the cell shape did not differ between studied groups after treatment.

### 3.5 Collagen type I synthesis and degradation in VSMCs

We performed RIA analyses to evaluate the synthesis and degradation of collagen type I. Synthesis (PINP) and degradation (ICTP) of collagen type I was similar between HGA patient and control cells.

# Discussion

## 1 General aspects

In this study, we have collected several HGA patient and control samples in co-operation with plastic surgeons at the Department of Plastic Surgery, Töölö Hospital of Helsinki University Hospital during 2010-2014. With this material we were able to perform different histological analyses to study new unknown aspects in HGA including the pathology of arteries, elastic fibres, collagen and smooth muscle cells. The findings in this study could be relevant even for other types amyloid angiopathy. We also have established *in vitro* cultured vascular smooth muscle cell lines. To the best of our knowledge these cell lines are unique in the world.

The total number of HGA patients in this study is 35 which can be consider rather high in a rare disease like HGA. Due to the rarity of the disease we were not able to collect different types of samples (tissues for several purposes and plasma) from the same HGA patients in order to compare the results from various analyses but on the other hand the results could be considered more reliable as the variation of the patient material is wider.

The research including sample collection from HGA patients and control subjects was approved by the ethical committee of HUH.

## 2 AGel amyloid angiopathy

The main findings in our study revealed extensive pathological changes in arteries of HGA patients. We observed severe disruption of the arterial wall with disorganization of VSMCs. In particular the *lamina elastica interna* was abnormally fragmented and diminished and VSMCs were disorganized leaving large areas of extracellular matrix between the cells in the *tunica media*. The disruption of the arterial wall and loss of elasticity causes very likely more fragile arteries with poor mechanical properties and abnormal vascular permeability which may contribute to some of the clinical findings and symptoms in HGA. Abnormal fragility has been demonstrated earlier clinically in the skin, especially in facial regions (Kiuru-Enari, Keski-Oja & Haltia, 2005, Pihlmaa et al., 2011), as well as in corneal tissue (Mattila et al., 2015). The

connection between *cutis laxa* and vascular wall vulnerability leading even to aorta ruptures, has been recognized in diseases caused by defects in the elastin gene leading to paucity of elastic fibres (Szabo et al., 2006).

The vascular permeability in HGA may cause increased intracutaneous as well as peri- and postoperative bleeding risk (Kiuru et al., 2000, Pihlmaa et al., 2011). In Finnish Gelsolin Amyloidosis Patient Registry (FIN-GAR) almost 70 % of the HGA patients reported intracutaneous, sometimes even very extensive bleedings only after normal physical contact, such as hugging or massage (Nikoskinen et al., 2015). Cerebral hemorrhages have also been reported, but the risk for those in HGA is not known presently. However, signs of abnormal vascular permeability, also in cerebral tissues, were observed in an HGA brain autopsy study (Kiuru, Salonen & Haltia, 1999), suggesting increased risk of cerebral hemorrhages. The pathological changes in arteries could also allow a better understanding of the characteristic neurodegeneration in HGA (Tanskanen et al., 2009), associated with AGel angiopathy in the brain, spinal cord, nerve roots and sensory ganglia as shown in previous neuropathological studies (Kiuru, Salonen & Haltia, 1999, Kiuru-Enari et al., 2002).

The loss of elastic fibers and elasticity in vascular walls most probably influences the circulation of blood as well by making it more challenging for the entire cardiovascular system. This could contribute to the higher consumption of cardiovascular medication in HGA patients (Laine et al., 2010). Hypertension is not a common feature in amyloidosis but renal vascular amyloidoses, including for example AA and AL amyloidoses, are often accompanied by arterial hypertension (Dember, 2006). This should be considered in HGA as well. Besides of the loss of elastic fibres, also the deposition of amyloid proteins in the ECM could lead to hypertension (Larsson et al., 2006).

The deposition of AGel fibrils was seen in both vascular and dermal tissues. The massive accumulation of fibrils, with a characteristic diameter of approximately 10 nm, was surrounding particularly elastic fibres. This finding is consistent with previous studies of other types of cutaneous amyloidosis (Winkelmann, Peters & Venencie, 1985, Sepp et al., 1990, Vecchietti et al., 2003, Bocquier et al., 2008, Marchand et al., 2013). Nevertheless, the amyloid fibril formation and accumulation in arteries does not seem to be severe enough to cause thrombosis in HGA patients.

Amyloid fibrils and microfibrils of elastic fibres fall into the same diameter range, approximately 10 nm (Braverman, Fonferko, 1982, Sipe, Cohen, 2000), which makes distinguishing of them very difficult based on the diameter measurement of a single filament in TEM. However, other factors like orientation, distribution pattern, and length of fibrils, indicate that the fibril matrix in vascular wall and skin of HGA patients was actually comprised of at least two different fibril types. Amyloid fibrils are typically disordered non-branching fibrils, appearing in oriented parallel forms only when occurring in massive amounts (Cohen, Calkins, 1959). Microfibrils of the elastic fibres form loosely packed parallel bundles (Kielty, Sherratt & Shuttleworth, 2002) and are well oriented, compared with amyloid fibrils located in deposits in ECM at other sites than elastic fibres. There is also a difference in the length of the fibrils; amyloid fibrils are only few micrometers long at most (Xue, Homans & Radford, 2009) while microfibrils can be longer depending on the underlying structures. Based on these morphological differences seen in TEM analyses, the fibril deposits along elastic fibers in HGA may comprise both amyloid fibrils and elastin-associated microfibrils.

Collagens have not been studied in HGA before, although findings at clinical examinations strongly suggest severe pathological derangement of connective tissues. Abnormal consistency and attachment of the skin to underlying tissues together with the difficulties in facial and cornea-transplant surgeries are reported (Pihlmaa et al., 2011, Mattila et al., 2015). We studied the presence, synthesis and degradation of collagen types I and III in arteries as they are the most abundant collagen types in arterial walls (Shekhonin et al., 1985). Our results showed that collagen types I and III were atypically focally accumulated in arteries, consistent with disruption of the *tunica media*. In arteries of HGA patients, collagen fibers seemed to re-localize to fill the empty space caused by degradation and disorganization of the arterial walls whereas in controls collagens were distributed evenly forming netlike patterns. Also, the degradation product of collagen type I (ICTP) was increased indicating problems in enzymatic activity or other abnormality of the clearing system.

### 3 Elastolysis and potential pathomechanisms behind it

Although the vascular system is often affected in systemic amyloidosis very little is known about the pathomechanisms in vessels (Kholova, Niessen, 2005). In arteries from HGA patients the *lamina elastica interna* was abnormally fragmented and diminished, sometimes even entirely absent, indicating serious elastolysis and causing severe disorganization of the arterial wall. Elastolysis in HGA has been described in the cutaneous tissue, particularly in the lower reticular dermis, proposed to contribute to cutis laxa (Kiuru-Enari, Keski-Oja & Haltia, 2005). These abnormalities typically increase with age and amount of amyloid, though the severity of the pathological changes may vary remarkably between patients in the same age group (Pihlmaa et al., 2016).

The elastolysis was very similar in HGA patients between the studied tissue types and between individuals, but it varied from the age-related elastolysis seen in control subjects. The main difference was the massive accumulation of fibrils surrounding fragmented elastic fibres in the samples of HGA patients, as in the control subjects no fibrils were observed. In addition, the degradation appeared more granular in control subjects, as shown in age-related elastolysis previously (Braverman, Fonferko, 1982, Pasquali-Ronchetti, Baccarani-Contrì, 1997). It seemed that elastin in particular is vulnerable to degradation, whereas the fibrillar matrix, consisting of both microfibrils and amyloid fibrils, is resistant to degradation. The same phenomenon was present in both dermal and vascular tissues. On the contrary, in degradation of elastic fibres in sun-damaged skin microfibrillar structures are lost (Suwabe et al., 1999). Our results might indicate that the microenvironment around elastic fibres in HGA favours both fibril accumulation and elastolysis.

The microenvironment around elastic fibres is complex because elastin is surrounded by a mantle of microfibrils comprising for example fibrillins, fibulins, emilins and GAGs (Hornebeck, Emonard, 2011). Elastases are able to adsorb rapidly onto elastin, but they can also bind onto cell surface-associated molecules such as GAGs. GAGs, on the other hand, are known to accelerate AGel fibril formation by hastening the fibril extension (Solomon et al., 2011). A high amount of GAGs may even permeate elastic fibres both in the skin and the aorta in lysyl oxidase (LOX) deficiency related connective tissue disorders (Pasquali-Ronchetti, Baccarani-Contrì, 1997). SAP could also participate in AGel accumulation and elastolysis. SAP which is

normally, also in healthy individuals, associated with elastic fibers (Breathnach et al., 1983), may form a nidus for amyloid deposition (Winkelmann, Peters & Venencie, 1985). SAP acts as an elastase inhibitor protecting not only normal elastic fibers but also pathological amyloid fibrils from proteolytic cleavages (Li, J. J., McAdam, 1984, Suwabe et al., 1999). Despite of the hypothetical protection by SAP, in HGA the elastic tissue is destroyed, whereas the AGel fibrils resist degradation. The ability of unsaturated fatty acids and heparin(s) to control elastase action has been delineated as well (Hornebeck, Emonard, 2011). The crucial factor concerning amyloid related elastolysis could be the immobilization and mechanical fatigue of elastin by amyloid fibrils which might create a microenvironment that favours elastolysis (Robert, Robert & Fulop, 2008, Sherratt, 2009, Hornebeck, Emonard, 2011).

Interestingly, the fibrous matrix can also include fibrils that are formed of elastin peptides. Elastin peptides are known to result from elastase-mediated elastin degradation also in normal conditions, such as aging (Baud et al., 2013). These peptides recruit inflammatory cells that release their elastases and thereby contribute to the generation of new elastin peptides. Their concentration in the blood flow is usually low but can reach high values in aged subjects, notably in those developing pathologies, where elastin is massively degraded (Baud et al., 2013). Unlike insoluble elastin, elastin peptides can modulate the physiology of numerous cells, such as fibroblasts, smooth muscle cells, endothelial cells, monocytes/macrophages, and lymphocytes (Baud et al., 2013). These bioactive peptides are termed elastokines or elastin-derived matrikines and they display dual biological functions in pathophysiology: they are potent inducers of protease expression catalysing collagenolysis or amplifying elastin degradation (Hornebeck, Emonard, 2011). This could be a possible phenomenon aggravating elastolysis in HGA as well. It is notable that elastin peptides are also able to form amyloid-like fibrils themselves (Bochicchio, Pepe & Tamburro, 2007, Bochicchio et al., 2013).

Searching for the potential pathomechanisms of elastolysis in HGA, we analysed several MMPs known to be capable of degrading insoluble elastic fibres: MMP-2 (72 kDa gelatinase), MMP-7 (matrilysin), MMP-9 (92 kDa gelatinase) and MMP-12 (macrophage metalloelastase) (Mecham et al., 1997) in tissue and plasma samples from HGA patients and controls. Our hypothesis was that AGel accumulation alongside elastic fibers may induce different enzymes, like MMPs, attempting to cleave pathological amyloid fibrils but unintentionally cleave elastin instead. Furthermore, it has been shown that VSMCs incubated together with medin fragments increase the production of MMP-2 which degrades elastin and collagen and

eventually weakens the vessel wall (Peng et al., 2007). We analysed also MMP-14, since it is considered to be responsible for the second cleavage of variant gelsolin resulting in the amyloidogenic fragments (5 and 8 kDa) (Page et al., 2005). We found that the levels of MMP-2, -7, -12 and -14 did not differ significantly between HGA patients and control subjects. However, MMP-9 levels were significantly lower in HGA patients than in controls. Interestingly, this same phenomenon has been reported also in Alzheimer's disease (Horstmann et al., 2010) and in fibrosis in end-stage human heart failure (Polyakova et al., 2011).

To understand the lower levels of MMP-9 in HGA patients we studied the previously reported cascade between MMP-9, TIMP-1 (inhibitor of MMP-9) and TGF $\beta$  (Akhurst, Hata, 2012, Kwak, 2013). TGF $\beta$ , often over-expressed in disease states, upregulates TIMP-1 gene expression which results in inhibition of MMP-9 (Akhurst, Hata, 2012, Kwak, 2013). TGF $\beta$  can also reduce elastin degradation by inhibiting MMP-9 activation by increasing the activities and/or levels of TIMP (Alvira et al., 2011). Unfortunately, the lower MMP-9 level was not explained with these cascades since the level of TIMP-1, an inhibitor of MMP-9, was not higher in HGA patients. However, TGF $\beta$  showed increased expression in HGA patients. Interestingly, TGF $\beta$  is associated with microfibrils, more precisely with fibrillin, in elastic fibres (Sherratt, 2009). Microfibrils even control the bioavailability of TGF $\beta$  in tissues (Massam-Wu et al., 2010). Furthermore, TGF $\beta$  is activated by MMP-2 and -9 (Leask, Abraham, 2004). A well-known function of TGF $\beta$  is to increase collagen synthesis and deposition by fibroblasts, leading to fibrosis, but it also promotes a proliferative and/or migratory phenotype of smooth muscle cells, which has a negative impact in some vascular diseases (Akhurst, Hata, 2012). TGF $\beta$  prevents amyloid induced degeneration (Fisichella et al., 2016) but may even contribute to the development of cerebrovascular amyloidosis (Weiss, Lifshitz & Frenkel, 2011). Apart from beneficial and deleterious roles in amyloidosis, TGF $\beta$  in line with HGA contributes to amyloidotic corneal lattice dystrophy (Han et al., 2011). In conclusion, TGF $\beta$  might play a functional role in AGel elastolysis even though fibrosis is not a known consequence in HGA.

The principal cause for the elastolysis remains to be studied further since the potential elastases (MMP-2, -7, -9, -12 and -14) were not shown to be expressed in higher amounts in HGA patients as in controls. It is to note that the amount of elastases does not necessarily correlate to their activities.



## 4 Biological effect of variant gelsolin in VSMCs

We established unique VSCM primary cell lines from the arteries of HGA patients and healthy controls to study the biological effect of variant gelsolin. Apart from platelets (Kiuru et al., 2000) and one fibroblast cell line (Paunio, T. et al., 1998), no other cell types isolated from HGA patients have been studied systematically so far. Platelets from HGA patients showed altered shape after stimulation with adenosine diphosphate and collagen compared to healthy controls (Kiuru et al., 2000). This may indicate that variant cytosolic gelsolin has an effect on actin cytoskeleton dynamics. Furthermore, in a neural cell line overexpression of gelsolin prevented the formation of actin stress fibers and transfection with variant gelsolin induced stabilization of F-actin and reduced plasticity of neural development (Westberg, Zhang & Andersson, 1999). In gelsolin null mice altered actin-cytosolic gelsolin interactions were demonstrated as the migration capacity of fibroblasts and neutrophils were reduced (Witke et al., 1995) and filopodia retraction was delayed *in vitro* (Lu et al., 1997). These previous results suggest that the variant cytosolic gelsolin and its possible malfunction might cause similar alterations in cells of HGA patients, and thereby possibly result in clinical symptoms and signs.

The expression of normal and variant gelsolin was confirmed by analyzing the mRNA sequence of HGA patient cell lines. Although both gene alleles were equally expressed in HGA patients on mRNA level, cells were not expressing variant gelsolin on protein level in a way that the C68 fragment of the gelsolin would have been detectable. We tried different methods to capture the C68 fragment: depletion of albumin from the media samples, use of serum free media in cell culture, immunoprecipitation and total protein enrichment to reduce high-abundance proteins and concentrate low-abundance proteins. The absence of the C68 fragment could be related to furin activity as well. The artificial environment in *in vitro* culture differs greatly from the natural three-dimensional environment in tissues which is filled with compounds and proteins regulating the others. Cells might need for example some signalling effect from other cells to support their normal behaviour. However, the absence of the C68 fragment is consistent with a previous study where only full-length gelsolin was found in the culture media of human fibroblasts transduced with recombinant adenoviruses encoding variant secretory gelsolin and from one HGA patient fibroblast cell line (Paunio, T. et al., 1998). In other types of transduced cells from kidney, lung, liver and, neuroectodermal origin

secreted C68 fragment in to culture media. The secretion of C68 might be cell type dependent. Overexpression experiments do not necessarily repeat the right conception of the behaviour of the cells in normal conditions as detected in patient cells.

With VSCM primary cell lines we focused to study the actin cytoskeleton organization and remodeling. On tissue level, in the arteries the morphology of individual VSMCs was normal in TEM analysis, but often the basal lamina of VSMCs was thickened. In cell culture VSMCs grew in an elongated way and formed orientated parallel bundles of cells which is the normal behaviour for these types of cells. The cells did not show any difference in migration ability between the studied groups. No dissimilarities in synthesis (PINP) and degradation (ICTP) of collagen type I were observed between HGA patient and control cells. Cells treated with PMA, a protein kinase C activator, behaved in the same way, although in a previous study, neural cells exposed to PMA responded very differently when variant and wild type gelsolin were overexpressed (Westberg, Zhang & Andersson, 1999). Neural cells expressing variant gelsolin produced extensive neurite outgrowths whereas cells expressing wild type gelsolin did not. The effect of staurosporine, a protein kinase C inhibitor, causing extreme stress and rapid disruption of actin microfilaments by inhibiting actin-cytosolic gelsolin induction (Miyamoto, Wu, 1990, Yu, Gotlieb, 1992) was studied too but the cell shape did not differ in VSMCs after treatment. As a conclusion, in *in vitro* studies no significant differences in actin cytoskeleton morphology or in cytosolic gelsolin expression were evident, when comparing cells lines from HGA patients and healthy controls. This is in line with a previous study which shows that the actin-modulating function was not impaired in mouse gelsolin-null fibroblasts transduced with variant gelsolin (Kangas et al., 1999). It is, however, quite surprising as gelsolin plays such a major role in cell migration and collagen metabolism (Sun et al., 1999, Li, G. H. et al., 2012).

It would have been very interesting to include cells from homozygous HGA patients in the study set to be able to compare possible distinctions of the cells. Yet, in heterozygous cells approximately half of the gelsolin, coded with the wild type allele, is functioning normally. On the other hand, there are even several other mechanisms that could compensate the possibly dysfunctional effect of the variant gelsolin. For example, all six members of gelsolin superfamily are known to regulate actin dynamics (Silacci et al., 2004). This is consistent with the notion that homozygote HGA patients, in spite earlier and more severe disease progression, did not seem to exhibit any unusual symptoms that could be attributed to a loss of function phenotype (Maury, 1993).

## 5 Candidates for biomarkers

Biomarkers are important diagnostic tools for a substantial number of different diseases or clinical conditions. They could be helpful also in therapeutic monitoring (Selleck, Senthil & Wall, 2017). Biomarkers have been under wide investigation in the researcher community and over the past 20 years, there has been an exponential increase in the number of biomarkers. Already by 2016 at least 768 259 published articles were directly related to biomarkers (Burke, 2016). As a comparison there are 896 articles directly related to gelsolin in PubMed to date. In HGA biomarkers would be helpful in the surveillance of a progression of the disease. Hopefully someday they could play a role in the drug discovery and therapeutic monitoring in HGA as well.

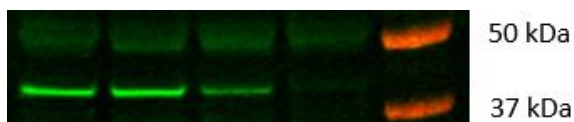
Gelsolin is a multifunctional protein with several activities along the actin homeostasis. Its role in a variety of physiological and pathobiological processes has led to the identification of gelsolin as a potential biomarker of inflammatory-related diseases. We have studied by large-scale proteomics a few potential biomarkers for HGA, of both gelsolin - and non-gelsolin based nature.

The following chapters include only unpublished data.

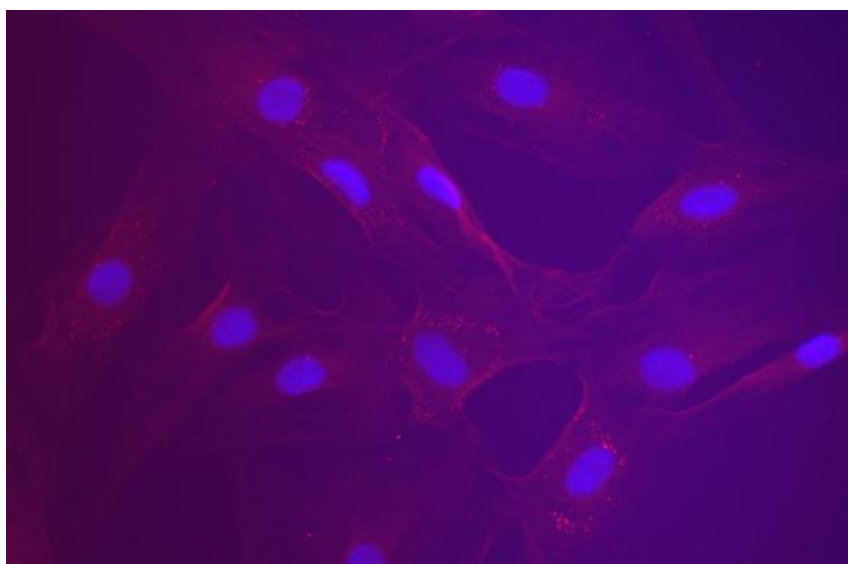
### 5.1 Alcohol dehydrogenase 1B

The proteome analysis was performed for VSMC primary cell lines using two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS) (Waters, Milford, MA, USA) which is a highly sensitive quantitative method for protein identification. Relative quantification between samples using precursor ion intensities was performed with Progenesis QI for Proteomics software (Nonlinear Dynamics/Waters) and ProteinLynx Global Server. Database searches were carried out against UniProtKB/Swiss-Prot. This study included VSMC cell lines from 7 HGA patients and 6 control subjects. Interestingly we found that alcohol dehydrogenase 1B (ADH1B) was upregulated in HGA patients cell lines by 6.7-fold compared to the control subject cell lines. The result was confirmed with Western blot analysis using Anti-ADH1B antibody (Thermo Fisher, Waltham, MA, USA) (Figure 14). The quantification of the Western blot was performed with infrared dye-based Odyssey imaging

combined with Image Studio software system (LI-COR, Lincoln, NE, USA). The results were impressively similar between two totally different methods as Western analysis showed upregulation of 7.4-fold in HGA patient cell lines compared to controls. The cells were also stained using the same antibody in immunocytochemistry as in Western blot analysis and it was evident that the cells were expressing ADH1B but the result was not quantitated (Figure 15).



**Figure 14** Western blot analysis of vascular smooth muscle cell lines using anti-ADH1B antibody. VSMCs show clear immunopositivity against ADH1B (green band sized 40 kDa) in VSMCs from HGA patients.



**Figure 15** VSMCs of HGA patient were expressing ADH1B (red dots). Nuclei are shown in blue (DAPI). Magnification x 400.

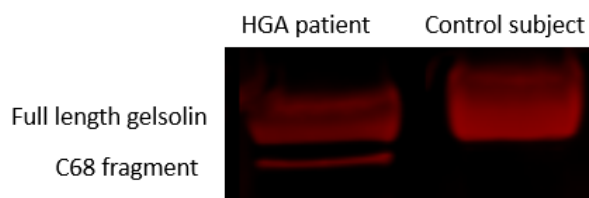
The members of alcohol dehydrogenase family metabolize several different substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. ADH1B exhibits high activity for ethanol oxidation and plays a major role in ethanol

catabolism. Generally, ADH1B and its different variants are related to alcohol consumption and dependence (Hart et al., 2016). For us it is still an open question how ADH1B relates to the molecular changes in cells and/or pathomechanisms in HGA. Thus, it would be very interesting to study further this protein as a biomarker for HGA. In Alzheimer's disease amyloid  $\beta$ -peptide-binding alcohol dehydrogenase (ABAD) has the capacity to bind amyloid  $\beta$ -peptide (Du Yan et al., 2000). ABAD belongs to the family of short chain dehydrogenase/reductases which have enzymatic activity toward a wide variety of substrates including n-isopropanol and  $\beta$ -estradiol. Maybe there is some binding mechanism and function also between ADH1B and A $\beta$  as has been shown for ABAD and A $\beta$ .

## **5.2 C68 fragment in tear fluid**

Another, well-known potential biomarker arises from the abnormal processing of the gelsolin protein during the maturation of the misfolded amyloid fragment. To study the gelsolin fragmentation in tear fluid we first performed a pilot study with two different kinds of tear fluid samples that were collected from a HGA patient; one included local anesthetic and the other one was native. Tear fluid was collected in a small capillary tubes and immediately frozen at -20°C. The analysis for gelsolin amyloid maturation compounds was performed by polyacrylamide gel electrophoresis under reducing conditions and Western blot analysis using a mouse monoclonal anti-gelsolin antibody (Sigma-Aldrich/Merck, Darmstadt, Germany). As a control we used recombinant C68 fragment (Van Overbeke et al., 2014). The result showed two separate bands on a gel in the HGA patient tear fluid: consistent with full length gelsolin and the C68 fragment.

The experiment was repeated with two HGA patient and two control subject samples following the same protocol. In controls the C68 band was not seen while in HGA patients both bands were evident (Figure 16).



**Figure 16** Western blot analysis of tear fluid using anti-gelsolin antibody. In the HGA patient the C68 fragment was clearly present whereas in the control subject it is absent.

Tear material for analyses, particularly in HGA where corneal lattice dystrophy plays a major role, would be ideal to be used since sample collection is noninvasive and a rather easy procedure. Therefore, the analysis of tear fluid and the results as presented here, are significant. In addition, tear fluid is much purer and less complex matrix to handle in different analyses compared to normal untreated blood (plasma and serum) samples where other high abundant proteins, especially albumin, affect the total protein analyses. Albumin concentration in tears is 0.023 mg/ml whereas in serum it is 53 mg/ml (Rauz, Saw, 2010). The albumin depletion from the blood samples, as usually done in blood-based proteomics experiments, does not necessarily solve the problem, because it may reduce also the amount and/or number of other proteins, as albumin is a carrier protein that binds to several other molecules leading to erroneous depletion of totally unrelated proteins. Considering the high sensitivity of the new generation of mass spectrometers we could easily foresee that such a technology could be used in the near future for the C68 fragment analysis from a little drop of tear fluid from HGA patients, creating the possibility for on-line non-invasive detection of disease progression.

## Conclusions and thoughts

- AGel amyloid angiopathy in HGA results in severe disruption of the arterial walls with massive AGel deposition. This may be responsible for several, particularly hemorrhagic, disease manifestations in HGA.
- Collagen derangement is consistent with clinical findings of pathological derangement of connective tissues. Collagens, in particular their degradation and clearing, are very likely linked to other pathological findings such as elastolysis and degeneration of the arterial wall.
- The accumulation of fibrils is associated with severe elastolysis. The elastolysis can be very striking leading to absence of elastic fibres. The loss of elasticity may cause considerable difficulties for HGA patients as the skin and the vascular system loses its functionality. The elastolysis could also appear in other organs as well which may have clinical significance in HGA.
- The fibrillar matrix is massive in HGA patients, especially surrounding elastic fibres like *lamina elastica interna*. This fibrillar matrix could consist of different kinds of fibrils: AGel amyloid fibrils, microfibrils of elastic fibres, and also amyloid-like fibrils consisting of elastin peptides.
- The microenvironment on elastic fibres seems to favour fibril accumulation and elastin degradation in HGA and in other reported amyloidoses with elastolysis. These microenvironmental changes could be differences in molecular conditions, for example presence of SAP and GAGs, but also the fibrils might accelerate fibril formation themselves. For example, the immobilization and mechanical fatigue of elastin by amyloid fibrils could influence fibril formation and accumulation.
- MMP-9 is decreased and TGF $\beta$  is increased in HGA patients. Reasons for these changes are yet to be revealed but reduced levels of MMP-9 have been seen in Alzheimer's

disease with deposition of A $\beta$  as well. There may be a regulating cascade existing that is involved in amyloid formation and existence at least in some amyloidoses.

- The unaltered cytoskeletal actin-gelsolin interactions in studied cells imply that HGA results rather from a toxic-gain-of-function than loss-of-function mechanism.
- Two potential biomarkers, alcohol dehydrogenase 1B (ADH1B) and the C68 fragment of variant gelsolin demonstrated in tear fluid of HGA patients, might be useful tools in evaluation of the progression of HGA in patient. They could possibly even aid in the follow-up of future specific treatment approaches.
- Amyloid angiopathy with severe elastolysis as observed in HGA, may have clinical significance even in other than AGel-related forms of amyloidosis.



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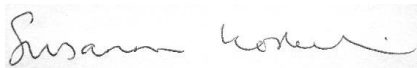
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Espoo 10<sup>th</sup> of August 2020

A handwritten signature in cursive script, reading "Susanna Koskelainen". The ink is dark and the handwriting is fluid, with a long horizontal stroke at the end.

Susanna Koskelainen

## References

- Abrahamson, M., Grubb, A., Olafsson, I. & Lundwall, A. 1987, "Molecular cloning and sequence analysis of cDNA coding for the precursor of the human cysteine proteinase inhibitor cystatin C", *FEBS letters*, vol. 216, no. 2, pp. 229-233.
- Aidinis, V., Carninci, P., Armaka, M., Witke, W., Harokopos, V., Pavelka, N., Koczan, D., Argyropoulos, C., Thwin, M.M., Moller, S., Waki, K., Gopalakrishnakone, P., Ricciardi-Castagnoli, P., Thiesen, H.J., Hayashizaki, Y. & Kollias, G. 2005, "Cytoskeletal rearrangements in synovial fibroblasts as a novel pathophysiological determinant of modeled rheumatoid arthritis", *PLoS genetics*, vol. 1, no. 4, pp. e48.
- Akhurst, R.J. & Hata, A. 2012, "Targeting the TGFbeta signalling pathway in disease", *Nature reviews.Drug discovery*, vol. 11, no. 10, pp. 790-811.
- Alabdali, M., Barnett, C., Abraham, A., Jaafari, D.A. & Bril, V. 2015, "Gelsolin Familial Amyloidosis Peripheral Neuropathy in Canada: A Case Report", *The Canadian journal of neurological sciences.Le journal canadien des sciences neurologiques*, vol. 42, no. 5, pp. 353-355.
- Alvira, C.M., Guignabert, C., Kim, Y.M., Chen, C., Wang, L., Duong, T.T., Yeung, R.S., Li, D.Y. & Rabinovitch, M. 2011, "Inhibition of transforming growth factor beta worsens elastin degradation in a murine model of Kawasaki disease", *The American journal of pathology*, vol. 178, no. 3, pp. 1210-1220.
- Anan, I., Kiuru-Enari, S., Obayashi, K., Ranlov, P.J. & Ando, Y. 2010, "Investigation of AGE, their receptor and NF-kappaB activation and apoptosis in patients with ATTR and Gelsolin amyloidosis", *Histology and histopathology*, vol. 25, no. 6, pp. 691-699.
- Ardalan, M.R., Shoja, M.M. & Kiuru-Enari, S. 2007, "Amyloidosis-related nephrotic syndrome due to a G654A gelsolin mutation: the first report from the Middle East", *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, vol. 22, no. 1, pp. 272-275.
- Asch, H.L., Head, K., Dong, Y., Natoli, F., Winston, J.S., Connolly, J.L. & Asch, B.B. 1996, "Widespread loss of gelsolin in breast cancers of humans, mice, and rats", *Cancer research*, vol. 56, no. 21, pp. 4841-4845.
- Atula, S., Nikoskinen, T., Saastamoinen, A., Strbian, D., Schmidt, E.K. & Kiuru-Enari, S. 2016, "Gender differences in the clinical course of Finnish gelsolin amyloidosis", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 23, no. 1, pp. 33-38.
- Bailey, A.J. 1978, "Collagen and elastin fibres", *Journal of Clinical Pathology*, vol. 31, Supplement (Royal College of Pathologists) 12, pp. 49-58.
- Baldwin, A.K., Simpson, A., Steer, R., Cain, S.A. & Kielty, C.M. 2013, "Elastic fibres in health and disease", *Expert reviews in molecular medicine*, vol. 15, pp. e8.
- Baud, S., Duca, L., Bochicchio, B., Brassart, B., Belloy, N., Pepe, A., Dauchez, M., Martiny, L. & Debelle, L. 2013, "Elastin peptides in aging and pathological conditions", *Biomolecular concepts*, vol. 4, no. 1, pp. 65-76.

- Bennett, M.R., Sinha, S. & Owens, G.K. 2016, "Vascular Smooth Muscle Cells in Atherosclerosis", *Circulation research*, vol. 118, no. 4, pp. 692-702.
- Benseny-Cases, N., Karamanos, T.K., Hoop, C.L., Baum, J. & Radford, S.E. 2019, "Extracellular matrix components modulate different stages in beta2-microglobulin amyloid formation", *The Journal of biological chemistry*, vol. 294, no. 24, pp. 9392-9401.
- Benson, M.D., Buxbaum, J.N., Eisenberg, D.S., Merlini, G., Saraiva, M.J.M., Sekijima, Y., Sipe, J.D. & Westermark, P. 2018, "Amyloid nomenclature 2018: recommendations by the International Society of Amyloidosis (ISA) nomenclature committee", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 25, no. 4, pp. 215-219.
- Benyamine, A., Bernard-Guervilly, F., Tummino, C., Macagno, N., Daniel, L., Valleix, S. & Granel, B. 2017, "Hereditary lysozyme amyloidosis with sicca syndrome, digestive, arterial, and tracheobronchial involvement: case-based review", *Clinical rheumatology*, vol. 36, no. 11, pp. 2623-2628.
- Bochicchio, B., Pepe, A., Delaunay, F., Lorusso, M., Baud, S. & Dauchez, M. 2013, "Amyloidogenesis of proteolytic fragments of human elastin", *RSC Advances*, vol. 3, pp. 13273-13285.
- Bochicchio, B., Pepe, A. & Tamburro, A. 2007, "Elastic fibers and amyloid deposition in vascular tissue", *Future Neurology*, vol. 2, no. 5, pp. 523-536.
- Bocquier, B., D'Incan, M., Joubert, J., Kanitakis, J., Salavert, M., Tournilhac, O., Maitre, F., Dechelotte, P., Souteyrand, P., Esteve, E. & Martin, L. 2008, "Amyloid elastosis: a new case studied extensively by electron microscopy and immunohistochemistry", *The British journal of dermatology*, vol. 158, no. 4, pp. 858-860.
- Boni, F., Milani, M., Barbiroli, A., Diomede, L., Mastrangelo, E. & de Rosa, M. 2018, "Gelsolin pathogenic Gly167Arg mutation promotes domain-swap dimerization of the protein", *Human molecular genetics*, vol. 27, no. 1, pp. 53-65.
- Boni, F., Milani, M., Porcari, R., Barbiroli, A., Ricagno, S. & de Rosa, M. 2016, "Molecular basis of a novel renal amyloidosis due to N184K gelsolin variant", *Scientific reports*, vol. 6, pp. 33463.
- Boraldi, F., Moscarelli, P., Bochicchio, B., Pepe, A., Salvi, A.M. & Quaglino, D. 2018, "Heparan sulfates facilitate harmless amyloidogenic fibril formation interacting with elastin-like peptides", *Scientific reports*, vol. 8, no. 1, pp. 3115-018-21472-0.
- Braverman, I.M. & Fonferko, E. 1982, "Studies in cutaneous aging: I. The elastic fiber network", *The Journal of investigative dermatology*, vol. 78, no. 5, pp. 434-443.
- Breathnach, S.M., Bhogal, B., de Beer, F.C., Melrose, S.M., Black, M.M. & Pepys, M.B. 1983, "Immunohistochemical studies of amyloid P component and fibronectin in erythropoietic protoporphyria", *The British journal of dermatology*, vol. 108, no. 3, pp. 267-275.
- Breathnach, S.M., Pepys, M.B. & Hintner, H. 1989, "Tissue amyloid P component in normal human dermis is non-covalently associated with elastic fiber microfibrils", *The Journal of investigative dermatology*, vol. 92, no. 1, pp. 53-58.
- Bucki, R., Levental, I., Kulakowska, A. & Janmey, P.A. 2008, "Plasma gelsolin: function, prognostic value, and potential therapeutic use", *Current Protein & Peptide Science*, vol. 9, no. 6, pp. 541-551.

- Budd, R.C., Firestein, G.S., Gabriel, S.E., Kelley, W.N., McInnes, I.B. & O'Dell, J.R. 2013, *Kelley's textbook of rheumatology*, 9th edn, Elsevier/Saunders, Philadelphia, PA.
- Bulut, M., Keles, N., Caliskan, Z., Kostek, O., Aksu, F., Ozdil, K., Akcakoyun, M., Demircioglu, K., Yilmaz, Y., Kanbay, M. & Caliskan, M. 2016, "Is the presence of AA amyloidosis associated with impaired coronary flow reserve?", *Atherosclerosis*, vol. 251, pp. 389-395.
- Burke, H.B. 2016, "Predicting Clinical Outcomes Using Molecular Biomarkers", *Biomarkers in cancer*, vol. 8, pp. 89-99.
- Burtnick, L.D., Koepf, E.K., Grimes, J., Jones, E.Y., Stuart, D.I., McLaughlin, P.J. & Robinson, R.C. 1997, "The crystal structure of plasma gelsolin: implications for actin severing, capping, and nucleation", *Cell*, vol. 90, no. 4, pp. 661-670.
- Burtnick, L.D., Robinson, R.C. & Choe, S. 2001, "Structure and function of gelsolin", *Results and problems in cell differentiation*, vol. 32, pp. 201-211.
- Burtnick, L.D., Urosev, D., Irobi, E., Narayan, K. & Robinson, R.C. 2004, "Structure of the N-terminal half of gelsolin bound to actin: roles in severing, apoptosis and FAF", *The EMBO journal*, vol. 23, no. 14, pp. 2713-2722.
- Bustamante, J.G. & Brito, D. 2017, "Amyloidosis" in *StatPearls* StatPearls Publishing LLC, Treasure Island (FL).
- Chaponnier, C., Borgia, R., Rungger-Brandle, E., Weil, R. & Gabbiani, G. 1979, "An actin-destabilizing factor is present in human plasma", *Experientia*, vol. 35, no. 8, pp. 1039-1041.
- Chauhan, V., Ji, L. & Chauhan, A. 2008, "Anti-amyloidogenic, anti-oxidant and anti-apoptotic role of gelsolin in Alzheimer's disease", *Biogerontology*, vol. 9, no. 6, pp. 381-389.
- Chen, C.D., Huff, M.E., Matteson, J., Page, L., Phillips, R., Kelly, J.W. & Balch, W.E. 2001, "Furin initiates gelsolin familial amyloidosis in the Golgi through a defect in Ca(2+) stabilization", *The EMBO journal*, vol. 20, no. 22, pp. 6277-6287.
- Choe, H., Burtnick, L.D., Mejillano, M., Yin, H.L., Robinson, R.C. & Choe, S. 2002, "The calcium activation of gelsolin: insights from the 3A structure of the G4-G6/actin complex", *Journal of Molecular Biology*, vol. 324, no. 4, pp. 691-702.
- Coelho, T., Merlini, G., Bulawa, C.E., Fleming, J.A., Judge, D.P., Kelly, J.W., Maurer, M.S., Plante-Bordeneuve, V., Labaudiniere, R., Mundayat, R., Riley, S., Lombardo, I. & Huertas, P. 2016, "Mechanism of Action and Clinical Application of Tafamidis in Hereditary Transthyretin Amyloidosis", *Neurology and therapy*, vol. 5, no. 1, pp. 1-25.
- Cohen, A.S. 1967, "Amyloidosis", *The New England journal of medicine*, vol. 277, pp. 522-530.
- Cohen, A.S. & Calkins, E. 1959, "Electron microscopic observations on a fibrous component in amyloid of diverse origins", *Nature*, vol. 183, no. 4669, pp. 1202-1203.
- Colby, D.W. & Prusiner, S.B. 2011, "Prions", *Cold Spring Harbor perspectives in biology*, vol. 3, no. 1, pp. a006833.
- Collinge, J. 2001, "Prion diseases of humans and animals: their causes and molecular basis", *Annual Review of Neuroscience*, vol. 24, pp. 519-550.

- Cooper, J.A., Loftus, D.J., Frieden, C., Bryan, J. & Elson, E.L. 1988, "Localization and mobility of gelsolin in cells", *The Journal of cell biology*, vol. 106, no. 4, pp. 1229-1240.
- Cornwell, G.G., 3rd & Westermark, P. 1980, "Senile amyloidosis: a protean manifestation of the aging process", *Journal of clinical pathology*, vol. 33, no. 12, pp. 1146-1152.
- De Corte, V., Gettemans, J. & Vandekerckhove, J. 1997, "Phosphatidylinositol 4,5-bisphosphate specifically stimulates PP60(c-src) catalyzed phosphorylation of gelsolin and related actin-binding proteins", *FEBS letters*, vol. 401, no. 2-3, pp. 191-196.
- de la Chapelle, A., Tolvanen, R., Boysen, G., Santavy, J., Bleeker-Wagemakers, L., Maury, C.P. & Kere, J. 1992, "Gelsolin-derived familial amyloidosis caused by asparagine or tyrosine substitution for aspartic acid at residue 187", *Nature genetics*, vol. 2, no. 2, pp. 157-160.
- Dember, L.M. 2006, "Amyloidosis-associated kidney disease", *Journal of the American Society of Nephrology : JASN*, vol. 17, no. 12, pp. 3458-3471.
- Dominguez, R. & Holmes, K.C. 2011, "Actin structure and function", *Annual review of biophysics*, vol. 40, pp. 169-186.
- Du Yan, S., Zhu, Y., Stern, E.D., Hwang, Y.C., Hori, O., Ogawa, S., Frosch, M.P., Connolly, E.S., Jr, McTaggart, R., Pinsky, D.J., Clarke, S., Stern, D.M. & Ramasamy, R. 2000, "Amyloid beta -peptide-binding alcohol dehydrogenase is a component of the cellular response to nutritional stress", *The Journal of biological chemistry*, vol. 275, no. 35, pp. 27100-27109.
- Eanes, E.D. & Glenner, G.G. 1968, "X-ray diffraction studies on amyloid filaments", *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*, vol. 16, no. 11, pp. 673-677.
- Eder, L., Zisman, D., Wolf, R. & Bitterman, H. 2007, "Pulmonary hypertension and amyloidosis--an uncommon association: a case report and review of the literature", *Journal of general internal medicine*, vol. 22, no. 3, pp. 416-419.
- Efebera, Y.A., Sturm, A., Baack, E.C., Hofmeister, C.C., Satoskar, A., Nadasdy, T., Nadasdy, G., Benson, D.M., Gillmore, J.D., Hawkins, P.N. & Rowczenio, D. 2014, "Novel gelsolin variant as the cause of nephrotic syndrome and renal amyloidosis in a large kindred", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 21, no. 2, pp. 110-112.
- Eriksson, M., Schonland, S., Yumlu, S., Hegenbart, U., von Hutten, H., Gioeva, Z., Lohse, P., Buttner, J., Schmidt, H. & Rocken, C. 2009, "Hereditary apolipoprotein AI-associated amyloidosis in surgical pathology specimens: identification of three novel mutations in the APOA1 gene", *The Journal of molecular diagnostics : JMD*, vol. 11, no. 3, pp. 257-262.
- Fatigati, V. & Murphy, R.A. 1984, "Actin and tropomyosin variants in smooth muscles. Dependence on tissue type", *The Journal of biological chemistry*, vol. 259, no. 23, pp. 14383-14388.
- Feng, L., Mejillano, M., Yin, H.L., Chen, J. & Prestwich, G.D. 2001, "Full-contact domain labeling: identification of a novel phosphoinositide binding site on gelsolin that requires the complete protein", *Biochemistry*, vol. 40, no. 4, pp. 904-913.

- Fisichella, V., Giurdanella, G., Platania, C.B., Romano, G.L., Leggio, G.M., Salomone, S., Drago, F., Caraci, F. & Bucolo, C. 2016, "TGF-beta1 prevents rat retinal insult induced by amyloid-beta (1-42) oligomers", *European journal of pharmacology*, vol. 787, pp. 72-77.
- Fock, U., Jockusch, B.M., Schubert, W.D. & Hinssen, H. 2005, "Topological assignment of the N-terminal extension of plasma gelsolin to the gelsolin surface", *The Biochemical journal*, vol. 385, no. Pt 3, pp. 659-665.
- Gabbiani, G., Schmid, E., Winter, S., Chaponnier, C., de Ckhashtonay, C., Vandekerckhove, J., Weber, K. & Franke, W.W. 1981, "Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific alpha-type actin", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 78, no. 1, pp. 298-302.
- Gales, L. 2019, "Tegsedi (Inotersen): An Antisense Oligonucleotide Approved for the Treatment of Adult Patients with Hereditary Transthyretin Amyloidosis", *Pharmaceuticals (Basel, Switzerland)*, vol. 12, no. 2, pp. 10.3390/ph12020078.
- Ghetti, B., Piccardo, P., Frangione, B., Bugianic, O., Giaccone, G., Young, K., Prelli, F., Farlowa, M., Dlouhy, S. & Tagliavini, F. 1996a, "Prion protein hereditary amyloidosis: parenchymal and vascular", *Seminars in Virology*, vol. 7, no. 3, pp. 189-200.
- Ghetti, B., Piccardo, P., Frangione, B., Bugiani, O., Giaccone, G., Young, K., Prelli, F., Farlow, M.R., Dlouhy, S.R. & Tagliavini, F. 1996b, "Prion protein amyloidosis", *Brain pathology (Zurich, Switzerland)*, vol. 6, no. 2, pp. 127-145.
- Ghiso, J. & Frangione, B. 2001, "Cerebral amyloidosis, amyloid angiopathy, and their relationship to stroke and dementia", *Journal of Alzheimer's disease : JAD*, vol. 3, no. 1, pp. 65-73.
- Ghiso, J., Haltia, M., Prelli, F., Novello, J. & Frangione, B. 1990, "Gelsolin variant (Asn-187) in familial amyloidosis, Finnish type", *The Biochemical journal*, vol. 272, no. 3, pp. 827-830.
- Ghiso, J.A., Holton, J., Miravalle, L., Calero, M., Lashley, T., Vidal, R., Houlden, H., Wood, N., Neubert, T.A., Rostagno, A., Plant, G., Revesz, T. & Frangione, B. 2001, "Systemic amyloid deposits in familial British dementia", *The Journal of biological chemistry*, vol. 276, no. 47, pp. 43909-43914.
- Gonzalez-Rodriguez, J., Ramirez-Miranda, A., Hernandez-Da Mota, S.E. & Zenteno, J.C. 2014, "TGFB1, CHST6, and GSN gene analysis in Mexican patients with stromal corneal dystrophies", *Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*, vol. 252, no. 8, pp. 1267-1272.
- Haddad, J.G., Harper, K.D., Guoth, M., Pietra, G.G. & Sanger, J.W. 1990, "Angiopathic consequences of saturating the plasma scavenger system for actin", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 4, pp. 1381-1385.
- Haggqvist, B., Naslund, J., Sletten, K., Westermark, G.T., Mucchiano, G., Tjernberg, L.O., Nordstedt, C., Engstrom, U. & Westermark, P. 1999, "Medin: an integral fragment of aortic smooth muscle cell-produced lactadherin forms the most common human amyloid", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 15, pp. 8669-8674.
- Haltia, M., Ghiso, J., Prelli, F., Gallo, G., Kiuru, S., Somer, H., Palo, J. & Frangione, B. 1990, "Amyloid in familial amyloidosis, Finnish type, is antigenically and structurally related to gelsolin", *The American journal of pathology*, vol. 136, no. 6, pp. 1223-1228.



- Han, Y.P., Sim, A.J., Vora, S.C. & Huang, A.J. 2011, "Unique TGFBI protein in lattice corneal dystrophy", *Investigative ophthalmology & visual science*, vol. 52, no. 11, pp. 8401-8406.
- Harris, D.A. & Schwartz, J.H. 1981, "Characterization of brevin, a serum protein that shortens actin filaments", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 78, no. 11, pp. 6798-6802.
- Harris, H.E. & Weeds, A.G. 1984, "Plasma gelsolin caps and severs actin filaments", *FEBS letters*, vol. 177, no. 2, pp. 184-188.
- Hart, A.B., Lynch, K.G., Farrer, L., Gelernter, J. & Kranzler, H.R. 2016, "Which alcohol use disorder criteria contribute to the association of ADH1B with alcohol dependence?", *Addiction Biology*, vol. 21, no. 4, pp. 924-938.
- Hawkins, P.N. & Pepys, M.B. 1995, "Imaging amyloidosis with radiolabelled SAP", *European journal of nuclear medicine*, vol. 22, no. 7, pp. 595-599.
- Hornebeck, W. & Emonard, H. 2011, "The cell-elastin-elastase(s) interacting triade directs elastolysis", *Frontiers in bioscience (Landmark edition)*, vol. 16, pp. 707-722.
- Horstmann, S., Budig, L., Gardner, H., Koziol, J., Deuschle, M., Schilling, C. & Wagner, S. 2010, "Matrix metalloproteinases in peripheral blood and cerebrospinal fluid in patients with Alzheimer's disease", *International psychogeriatrics*, vol. 22, no. 6, pp. 966-972.
- Horvath-Szalai, Z., Kustan, P., Szirmay, B., Lakatos, A., Christensen, P.H., Huber, T., Bugyi, B., Muhl, D., Ludany, A., Miseta, A., Kovacs, G.L. & Koszegi, T. 2017, "Validation of an automated immune turbidimetric assay for serum gelsolin and its possible clinical utility in sepsis", *Journal of clinical laboratory analysis*, .
- House, S.J., Potier, M., Bisailon, J., Singer, H.A. & Trebak, M. 2008, "The non-excitable smooth muscle: calcium signaling and phenotypic switching during vascular disease", *Pflugers Archiv : European journal of physiology*, vol. 456, no. 5, pp. 769-785.
- Huff, M.E., Page, L.J., Balch, W.E. & Kelly, J.W. 2003, "Gelsolin domain 2 Ca<sup>2+</sup> affinity determines susceptibility to furin proteolysis and familial amyloidosis of finnish type", *Journal of Molecular Biology*, vol. 334, no. 1, pp. 119-127.
- Iannuzzi, C., Irace, G. & Sirangelo, I. 2015, "The effect of glycosaminoglycans (GAGs) on amyloid aggregation and toxicity", *Molecules (Basel, Switzerland)*, vol. 20, no. 2, pp. 2510-2528.
- Janmey, P.A., Chaponnier, C., Lind, S.E., Zaner, K.S., Stossel, T.P. & Yin, H.L. 1985, "Interactions of gelsolin and gelsolin-actin complexes with actin. Effects of calcium on actin nucleation, filament severing, and end blocking", *Biochemistry*, vol. 24, no. 14, pp. 3714-3723.
- Janmey, P.A. & Stossel, T.P. 1987, "Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate", *Nature*, vol. 325, no. 6102, pp. 362-364.
- Jansen, C., Parchi, P., Capellari, S., Vermeij, A.J., Corrado, P., Baas, F., Strammiello, R., van Gool, W.A., van Swieten, J.C. & Rozemuller, A.J. 2010, "Prion protein amyloidosis with divergent phenotype associated with two novel nonsense mutations in PRNP", *Acta Neuropathologica*, vol. 119, no. 2, pp. 189-197.

- Ji, L., Chauhan, A., Wegiel, J., Essa, M.M. & Chauhan, V. 2009, "Gelsolin is proteolytically cleaved in the brains of individuals with Alzheimer's disease", *Journal of Alzheimer's disease : JAD*, vol. 18, no. 1, pp. 105-111.
- Johansson, B., Wernstedt, C. & Westermark, P. 1987, "Atrial natriuretic peptide deposited as atrial amyloid fibrils", *Biochemical and biophysical research communications*, vol. 148, no. 3, pp. 1087-1092.
- Juusela, P., Tanskanen, M., Nieminen, A., Kari, K., Suominen, L., Uitto, V.J. & Kiuru-Enari, S. 2013, "Xerostomia in hereditary gelsolin amyloidosis", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 20, no. 1, pp. 39-44.
- Juusela, P., Tanskanen, M., Nieminen, A., Uitto, V.J., Blafield, H. & Kiuru-Enari, S. 2009, "Hereditary gelsolin amyloidosis mimicking Sjogren's syndrome", *Clinical rheumatology*, vol. 28, no. 11, pp. 1351-1354.
- Juusela, P.L., Persson, R.G., Nieminen, A.R., Kiuru-Enari, S.M. & Uitto, V.J. 2015, "Relation of gelsolin amyloidosis and periodontal health", *Clinical oral investigations*, vol. 19, no. 2, pp. 229-235.
- Kaneko, S. & Yamagata, K. 2018, "Hemodialysis-related amyloidosis: Is it still relevant?", *Seminars in dialysis*, vol. 31, no. 6, pp. 612-618.
- Kangas, H., Paunio, T., Kalkkinen, N., Jalanko, A. & Peltonen, L. 1996, "In vitro expression analysis shows that the secretory form of gelsolin is the sole source of amyloid in gelsolin-related amyloidosis", *Human molecular genetics*, vol. 5, no. 9, pp. 1237-1243.
- Kangas, H., Seidah, N.G. & Paunio, T. 2002, "Role of proprotein convertases in the pathogenic processing of the amyloidosis-associated form of secretory gelsolin", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 9, no. 2, pp. 83-87.
- Kangas, H., Ulmanen, I., Paunio, T., Kwiatkowski, D.J., Lehtovirta, M., Jalanko, A. & Peltonen, L. 1999, "Functional consequences of amyloidosis mutation for gelsolin polypeptide -- analysis of gelsolin-actin interaction and gelsolin processing in gelsolin knock-out fibroblasts", *FEBS letters*, vol. 454, no. 3, pp. 233-239.
- Kantanen, M., Kiuru-Enari, S., Salonen, O., Kaipainen, M. & Hokkanen, L. 2014, "Subtle neuropsychiatric and neurocognitive changes in hereditary gelsolin amyloidosis (AGel amyloidosis)", *PeerJ*, vol. 2, pp. e493.
- Kapoor, M., Rossor, A.M., Laura, M. & Reilly, M.M. 2019, "Clinical Presentation, Diagnosis and Treatment of TTR Amyloidosis", *Journal of neuromuscular diseases*, vol. 6, no. 2, pp. 189-199.
- Kawaji, T., Ando, Y., Nakamura, M., Yamashita, T., Wakita, M., Ando, E., Hirata, A. & Tanihara, H. 2005, "Ocular amyloid angiopathy associated with familial amyloidotic polyneuropathy caused by amyloidogenic transthyretin Y114C", *Ophthalmology*, vol. 112, no. 12, pp. 2212.
- Kawamura, S., Takahashi, M., Ishihara, T. & Uchino, F. 1995, "Incidence and distribution of isolated atrial amyloid: histologic and immunohistochemical studies of 100 aging hearts", *Pathology international*, vol. 45, no. 5, pp. 335-342.

- Kholova, I. & Niessen, H.W. 2005, "Amyloid in the cardiovascular system: a review", *Journal of clinical pathology*, vol. 58, no. 2, pp. 125-133.
- Kielty, C.M., Sherratt, M.J. & Shuttleworth, C.A. 2002, "Elastic fibres", *Journal of cell science*, vol. 115, no. Pt 14, pp. 2817-2828.
- Kiuru, S. 1998, "Gelsolin-related familial amyloidosis, Finnish type (FAF), and its variants found worldwide", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 5, no. 1, pp. 55-66.
- Kiuru, S. 1992, "Familial amyloidosis of the Finnish type (FAF). A clinical study of 30 patients", *Acta Neurologica Scandinavica*, vol. 86, no. 4, pp. 346-353.
- Kiuru, S., Javela, K., Somer, H. & Kekomaki, R. 2000, "Altered platelet shape change in hereditary gelsolin Asp187Asn-related amyloidosis", *Thrombosis and haemostasis*, vol. 83, no. 3, pp. 491-495.
- Kiuru, S., Matikainen, E., Kupari, M., Haltia, M. & Palo, J. 1994, "Autonomic nervous system and cardiac involvement in familial amyloidosis, Finnish type (FAF)", *Journal of the neurological sciences*, vol. 126, no. 1, pp. 40-48.
- Kiuru, S., Nieminen, T. & Partinen, M. 1999, "Obstructive sleep apnoea syndrome in hereditary gelsolin-related amyloidosis", *Journal of sleep research*, vol. 8, no. 2, pp. 143-149.
- Kiuru, S., Salonen, O. & Haltia, M. 1999, "Gelsolin-related spinal and cerebral amyloid angiopathy", *Annals of Neurology*, vol. 45, no. 3, pp. 305-311.
- Kiuru, S., Seppäläinen, A., Salonen, O., Hokkanen, L., Somer, H. & Palo, J. 1995, "CNS abnormalities in patients with familial amyloidosis, Finnish type (FAF)", *Amyloid*, vol. Vol. 2, No. 1, pp. 22-30.
- Kiuru, S. & Seppäläinen, A.M. 1994, "Neuropathy in familial amyloidosis , Finnish type (FAF): electrophysiological studies", *Muscle & nerve*, vol. 17, no. 3, pp. 299-304.
- Kiuru-Enari, S. & Haltia, M. 2013, "Hereditary gelsolin amyloidosis", *Handbook of clinical neurology*, vol. 115, pp. 659-681.
- Kiuru-Enari, S., Keski-Oja, J. & Haltia, M. 2005, "Cutis laxa in hereditary gelsolin amyloidosis", *The British journal of dermatology*, vol. 152, no. 2, pp. 250-257.
- Kiuru-Enari, S., Somer, H., Seppäläinen, A.M., Notkola, I.L. & Haltia, M. 2002, "Neuromuscular pathology in hereditary gelsolin amyloidosis", *Journal of neuropathology and experimental neurology*, vol. 61, no. 6, pp. 565-571.
- Kivelä, T., Tarkkanen, A., Frangione, B., Ghiso, J. & Haltia, M. 1994, "Ocular amyloid deposition in familial amyloidosis, Finnish: an analysis of native and variant gelsolin in Meretoja's syndrome", *Investigative ophthalmology & visual science*, vol. 35, no. 10, pp. 3759-3769.
- Koike, H. & Katsuno, M. 2019, "Ultrastructure in Transthyretin Amyloidosis: From Pathophysiology to Therapeutic Insights", *Biomedicines*, vol. 7, no. 1, pp. 10.3390/biomedicines7010011.
- Korkmaz, B., Horwitz, M.S., Jenne, D.E. & Gauthier, F. 2010, "Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases", *Pharmacological reviews*, vol. 62, no. 4, pp. 726-759.

- Kothakota, S., Azuma, T., Reinhard, C., Klippel, A., Tang, J., Chu, K., McGarry, T.J., Kirschner, M.W., Kohts, K., Kwiatkowski, D.J. & Williams, L.T. 1997, "Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis", *Science (New York, N.Y.)*, vol. 278, no. 5336, pp. 294-298.
- Kunecki, M. & Nawrocka, A. 2001, "Elastin-laminin receptor and abdominal aortic aneurysms. New subject to study? A review", *Pathologie-biologie*, vol. 49, no. 4, pp. 333-338.
- Kuzumaki, N., Tanaka, M., Sakai, N. & Fujita, H. 1997, "Tumor suppressive function of gelsolin", *Gan to kagaku ryoho.Cancer & chemotherapy*, vol. 24, no. 11, pp. 1436-1441.
- Kwak, H.B. 2013, "Aging, exercise, and extracellular matrix in the heart", *Journal of exercise rehabilitation*, vol. 9, no. 3, pp. 338-347.
- Kwiatkowski, D.J. 1999, "Functions of gelsolin: motility, signaling, apoptosis, cancer", *Current opinion in cell biology*, vol. 11, no. 1, pp. 103-108.
- Kwiatkowski, D.J., Janmey, P.A. & Yin, H.L. 1989, "Identification of critical functional and regulatory domains in gelsolin", *The Journal of cell biology*, vol. 108, no. 5, pp. 1717-1726.
- Kwiatkowski, D.J., Mehl, R., Izumo, S., Nadal-Ginard, B. & Yin, H.L. 1988, "Muscle is the major source of plasma gelsolin", *The Journal of biological chemistry*, vol. 263, no. 17, pp. 8239-8243.
- Kwiatkowski, D.J., Mehl, R. & Yin, H.L. 1988, "Genomic organization and biosynthesis of secreted and cytoplasmic forms of gelsolin", *The Journal of cell biology*, vol. 106, no. 2, pp. 375-384.
- Kwiatkowski, D.J., Stossel, T.P., Orkin, S.H., Mole, J.E., Colten, H.R. & Yin, H.L. 1986, "Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain", *Nature*, vol. 323, no. 6087, pp. 455-458.
- Kwiatkowski, D.J., Westbrook, C.A., Bruns, G.A. & Morton, C.C. 1988, "Localization of gelsolin proximal to ABL on chromosome 9", *American Journal of Human Genetics*, vol. 42, no. 4, pp. 565-572.
- LaFerla, F.M., Green, K.N. & Oddo, S. 2007, "Intracellular amyloid-beta in Alzheimer's disease", *Nature reviews.Neuroscience*, vol. 8, no. 7, pp. 499-509.
- Laine, A., Kääriäinen, H., Notkola, I. & Kiuru-Enari, S. 2010, "Perinnöllinen Meretojan tauti. Runsas oirekirjo heikentää elämänlaatua ja lisää terveyspalvelujen käyttöä.", *Suomen Lääkärilehti*, vol. 16, pp. 1417-1424.
- Lamb, J.A., Allen, P.G., Tuan, B.Y. & Janmey, P.A. 1993, "Modulation of gelsolin function. Activation at low pH overrides Ca<sup>2+</sup> requirement", *The Journal of biological chemistry*, vol. 268, no. 12, pp. 8999-9004.
- Larsson, A., Peng, S., Persson, H., Rosenbloom, J., Abrams, W.R., Wassberg, E., Thelin, S., Sletten, K., Gerwins, P. & Westermark, P. 2006, "Lactadherin binds to elastin--a starting point for medin amyloid formation?", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 13, no. 2, pp. 78-85.
- Leask, A. & Abraham, D.J. 2004, "TGF-beta signaling and the fibrotic response", *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, vol. 18, no. 7, pp. 816-827.

- Lee, R.M., Owens, G.K., Scott-Burden, T., Head, R.J., Mulvany, M.J. & Schiffrin, E.L. 1995, "Pathophysiology of smooth muscle in hypertension", *Canadian journal of physiology and pharmacology*, vol. 73, no. 5, pp. 574-584.
- Levy, E., Haltia, M., Fernandez-Madrid, I., Koivunen, O., Ghiso, J., Prelli, F. & Frangione, B. 1990, "Mutation in gelsolin gene in Finnish hereditary amyloidosis", *The Journal of experimental medicine*, vol. 172, no. 6, pp. 1865-1867.
- Li, G.H., Arora, P.D., Chen, Y., McCulloch, C.A. & Liu, P. 2012, "Multifunctional roles of gelsolin in health and diseases", *Medicinal research reviews*, vol. 32, no. 5, pp. 999-1025.
- Li, J.J. & McAdam, K.P. 1984, "Human amyloid P component: an elastase inhibitor", *Scandinavian Journal of Immunology*, vol. 20, no. 3, pp. 219-226.
- Li, Z., Xu, H., Liu, D., Li, D., Liu, G. & Wang, S.X. 2019, "Hereditary renal amyloidosis with a variant lysozyme p.Trp82Arg in a Chinese family: case report and literature review", *BMC nephrology*, vol. 20, no. 1, pp. 310-019-1496-6.
- Liang, C.C., Park, A.Y. & Guan, J.L. 2007, "In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro", *Nature protocols*, vol. 2, no. 2, pp. 329-333.
- Li-ChunHsieh, K., Schob, S., Zeller, M.W., Pulli, B., Ali, M., Wang, C., Chiou, T.T., Tsang, Y.M., Lee, P.S., Stossel, T.P. & Chen, J.W. 2015, "Gelsolin decreases actin toxicity and inflammation in murine multiple sclerosis", *Journal of neuroimmunology*, vol. 287, pp. 36-42.
- Lin, K.M., Mejillano, M. & Yin, H.L. 2000, "Ca<sup>2+</sup> regulation of gelsolin by its C-terminal tail", *The Journal of biological chemistry*, vol. 275, no. 36, pp. 27746-27752.
- Lin, K.M., Wenegieme, E., Lu, P.J., Chen, C.S. & Yin, H.L. 1997, "Gelsolin binding to phosphatidylinositol 4,5-bisphosphate is modulated by calcium and pH", *The Journal of biological chemistry*, vol. 272, no. 33, pp. 20443-20450.
- Lind, S.E., Yin, H.L. & Stossel, T.P. 1982, "Human platelets contain gelsolin. A regulator of actin filament length", *The Journal of clinical investigation*, vol. 69, no. 6, pp. 1384-1387.
- Linke, R.P. 2012, "On typing amyloidosis using immunohistochemistry. Detailed illustrations, review and a note on mass spectrometry", *Progress in histochemistry and cytochemistry*, vol. 47, no. 2, pp. 61-132.
- Liu, C.C., Zhao, N., Fu, Y., Wang, N., Linares, C., Tsai, C.W. & Bu, G. 2017, "ApoE4 Accelerates Early Seeding of Amyloid Pathology", *Neuron*, vol. 96, no. 5, pp. 1024-1032.e3.
- Lu, M., Witke, W., Kwiatkowski, D.J. & Kosik, K.S. 1997, "Delayed retraction of filopodia in gelsolin null mice", *The Journal of cell biology*, vol. 138, no. 6, pp. 1279-1287.
- Lucero Saa, F., Cremona, F.A., Minguez, N.X., Igarzabal, M.L. & Chiaradia, P. 2017, "The First Argentinian Family with Familial Amyloidosis of the Finnish Type", *Case reports in ophthalmology*, vol. 8, no. 2, pp. 446-451.
- Lueck, A., Brown, D. & Kwiatkowski, D.J. 1998, "The actin-binding proteins adseverin and gelsolin are both highly expressed but differentially localized in kidney and intestine", *Journal of Cell Science*, vol. 111, pp. 3633-3643.

- Lueck, A., Yin, H.L., Kwiatkowski, D.J. & Allen, P.G. 2000, "Calcium regulation of gelsolin and adseverin: a natural test of the helix latch hypothesis", *Biochemistry*, vol. 39, no. 18, pp. 5274-5279.
- Mahalka, A.K., Maury, C.P. & Kinnunen, P.K. 2011, "1-Palmitoyl-2-(9'-oxononanoyl)-sn-glycero-3-phosphocholine, an oxidized phospholipid, accelerates Finnish type familial gelsolin amyloidosis in vitro", *Biochemistry*, vol. 50, no. 22, pp. 4877-4889.
- Manabe, S., Hatano, M., Yazaki, M., Nitta, K. & Nagata, M. 2015, "Renal AH Amyloidosis Associated With a Truncated Immunoglobulin Heavy Chain Undetectable by Immunostaining", *American Journal of Kidney Diseases : The Official Journal of the National Kidney Foundation*, vol. 66, no. 6, pp. 1095-1100.
- Maramattom, B.V. & Chickabasaviah, Y.T. 2013, "A new Indian family affected by gelsolin amyloidosis", *Neurology India*, vol. 61, no. 6, pp. 673-675.
- Marchand, A., Levaltier, X., Croue, A., Arbeille, B., Ifrah, N. & Martin, L. 2013, "Cutaneous amyloid elastosis revealing multiple myeloma with systemic amyloidosis", *Acta Dermato-Venereologica*, vol. 93, no. 2, pp. 204-205.
- Massam-Wu, T., Chiu, M., Choudhury, R., Chaudhry, S.S., Baldwin, A.K., McGovern, A., Baldock, C., Shuttleworth, C.A. & Kielty, C.M. 2010, "Assembly of fibrillin microfibrils governs extracellular deposition of latent TGF beta", *Journal of cell science*, vol. 123, no. Pt 17, pp. 3006-3018.
- Mattila, J.S., Krootila, K., Kivela, T. & Holopainen, J.M. 2015, "Penetrating keratoplasty for corneal amyloidosis in familial amyloidosis, Finnish type", *Ophthalmology*, vol. 122, no. 3, pp. 457-463.
- Maury, C.P. 1993, "Homozygous familial amyloidosis, Finnish type: demonstration of glomerular gelsolin-derived amyloid and non-amyloid tubular gelsolin", *Clinical nephrology*, vol. 40, no. 1, pp. 53-56.
- Maury, C.P. 1991, "Gelsolin-related amyloidosis. Identification of the amyloid protein in Finnish hereditary amyloidosis as a fragment of variant gelsolin", *The Journal of clinical investigation*, vol. 87, no. 4, pp. 1195-1199.
- Maury, C.P., Kere, J., Tolvanen, R. & de la Chapelle, A. 1992, "Homozygosity for the Asn187 gelsolin mutation in Finnish-type familial amyloidosis is associated with severe renal disease", *Genomics*, vol. 13, no. 3, pp. 902-903.
- Maury, C.P., Kere, J., Tolvanen, R. & de la Chapelle, A. 1990, "Finnish hereditary amyloidosis is caused by a single nucleotide substitution in the gelsolin gene", *FEBS letters*, vol. 276, no. 1-2, pp. 75-77.
- Maury, C.P. & Rossi, H. 1993, "Demonstration of a circulating 65K gelsolin variant specific for familial amyloidosis, Finnish type", *Biochemical and biophysical research communications*, vol. 191, no. 1, pp. 41-44.
- Mecham, R.P., Broekelmann, T.J., Fliszar, C.J., Shapiro, S.D., Welgus, H.G. & Senior, R.M. 1997, "Elastin degradation by matrix metalloproteinases. Cleavage site specificity and mechanisms of elastolysis", *The Journal of biological chemistry*, vol. 272, no. 29, pp. 18071-18076.
- Meretoja, J. 1976, "Tautiperintöämme: suomalainen amyloiditauti. Matti Orava ja 253 potilasta.", *Suomen Lääkärilehti*, , no. 31, pp. 2234-2236.

- Meretoja, J. 1973, "Genetic aspects of familial amyloidosis with corneal lattice dystrophy and cranial neuropathy", *Clinical genetics*, vol. 4, no. 3, pp. 173-185.
- Meretoja, J. 1969, "Familial systemic paramyloidosis with lattice dystrophy of the cornea, progressive cranial neuropathy, skin changes and various internal symptoms. A previously unrecognized heritable syndrome", *Annals of Clinical Research*, vol. 1, no. 4, pp. 314-324.
- Meretoja, J. & Teppo, L. 1971, "Histopathological findings of familial amyloidosis with cranial neuropathy as principal manifestation. Report on three cases", *Acta pathologica et microbiologica Scandinavica. Section A, Pathology*, vol. 79, no. 5, pp. 432-440.
- Merlini, G. 2017, "AL amyloidosis: from molecular mechanisms to targeted therapies", *Hematology. American Society of Hematology. Education Program*, vol. 2017, no. 1, pp. 1-12.
- Metz, R.P., Patterson, J.L. & Wilson, E. 2012, "Vascular smooth muscle cells: isolation, culture, and characterization", *Methods in molecular biology (Clifton, N.J.)*, vol. 843, pp. 169-176.
- Michel, J.B., Li, Z. & Lacolley, P. 2012, "Smooth muscle cells and vascular diseases", *Cardiovascular research*, vol. 95, no. 2, pp. 135-137.
- Mikami, M., Zhang, Y., Danielsson, J., Joell, T., Yong, H.M., Townsend, E., Khurana, S., An, S.S. & Emala, C.W. 2017, "Impaired Relaxation of Airway Smooth Muscle in Mice Lacking the Actin-Binding Protein Gelsolin", *American journal of respiratory cell and molecular biology*, vol. 56, no. 5, pp. 628-636.
- Miyamoto, S. & Wu, J.M. 1990, "Effect of staurosporine on the induction of actin/gelsolin in PMA-treated HL-60 cells", *Biochemistry international*, vol. 22, no. 3, pp. 427-433.
- Modesto, K.M., Dispenzieri, A., Gertz, M., Cauduro, S.A., Khandheria, B.K., Seward, J.B., Kyle, R., Wood, C.M., Bailey, K.R., Tajik, A.J., Miller, F.A., Pellikka, P.A. & Abraham, T.P. 2007, "Vascular abnormalities in primary amyloidosis", *European heart journal*, vol. 28, no. 8, pp. 1019-1024.
- Moutafi, M., Ziogas, D.C., Michopoulos, S., Bagratuni, T., Vasileiou, V., Verga, L., Merlini, G., Palladini, G., Matsouka, C., Dimopoulos, M.A. & Kastritis, E. 2019, "A new genetic variant of hereditary apolipoprotein A-I amyloidosis: a case-report followed by discussion of diagnostic challenges and therapeutic options", *BMC medical genetics*, vol. 20, no. 1, pp. 23-019-0755-5.
- Mucchiano, G., Cornwell, G.G., 3rd & Westermark, P. 1992, "Senile aortic amyloid. Evidence for two distinct forms of localized deposits", *The American journal of pathology*, vol. 140, no. 4, pp. 871-877.
- Mustonen, T., Schmidt, E.K., Valori, M., Tienari, P.J., Atula, S. & Kiuru-Enari, S. 2018, "Common origin of the gelsolin gene variant in 62 Finnish AGel amyloidosis families", *European journal of human genetics : EJHG*, vol. 26, no. 1, pp. 117-123.
- Nag, S., Larsson, M., Robinson, R.C. & Burtnick, L.D. 2013, "Gelsolin: the tail of a molecular gymnast", *Cytoskeleton (Hoboken, N.J.)*, vol. 70, no. 7, pp. 360-384.
- Nag, S., Ma, Q., Wang, H., Chumnarnsilpa, S., Lee, W.L., Larsson, M., Kannan, B., Hernandez-Valladares, M., Burtnick, L.D. & Robinson, R.C. 2009, "Ca<sup>2+</sup> binding by domain 2 plays a critical role in the activation and stabilization of gelsolin", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 33, pp. 13713-13718.

- Nikoskinen, T., Schmidt, E.K., Strbian, D., Kiuru-Enari, S. & Atula, S. 2015, "Natural course of Finnish gelsolin amyloidosis", *Annals of Medicine*, vol. 47, no. 6, pp. 506-511.
- Norero, B., Perez-Ayuso, R.M., Duarte, I., Ramirez, P., Soza, A., Arrese, M. & Benitez, C. 2013, "Portal hypertension and acute liver failure as uncommon manifestations of primary amyloidosis", *Annals of hepatology*, vol. 13, no. 1, pp. 142-149.
- Norio, R. 2003, "The Finnish Disease Heritage III: the individual diseases", *Human genetics*, vol. 112, no. 5-6, pp. 470-526.
- Ohtsu, M., Sakai, N., Fujita, H., Kashiwagi, M., Gasa, S., Shimizu, S., Eguchi, Y., Tsujimoto, Y., Sakiyama, Y., Kobayashi, K. & Kuzumaki, N. 1997, "Inhibition of apoptosis by the actin-regulatory protein gelsolin", *The EMBO journal*, vol. 16, no. 15, pp. 4650-4656.
- Oregel, K.Z., Shouse, G.P., Oster, C., Martinez, F., Wang, J., Rosenzweig, M., Deisch, J.K., Chen, C.S. & Nagaraj, G. 2018, "Atypical Presentation of Gelsolin Amyloidosis in a Man of African Descent with a Novel Mutation in the Gelsolin Gene", *The American journal of case reports*, vol. 19, pp. 374-381.
- Owens, G.K. 1995, "Regulation of differentiation of vascular smooth muscle cells", *Physiological Reviews*, vol. 75, no. 3, pp. 487-517.
- Page, L.J., Huff, M.E., Kelly, J.W. & Balch, W.E. 2004, "Ca<sup>2+</sup> binding protects against gelsolin amyloidosis", *Biochemical and biophysical research communications*, vol. 322, no. 4, pp. 1105-1110.
- Page, L.J., Suk, J.Y., Huff, M.E., Lim, H.J., Venable, J., Yates, J., Kelly, J.W. & Balch, W.E. 2005, "Metalloendoprotease cleavage triggers gelsolin amyloidogenesis", *The EMBO journal*, vol. 24, no. 23, pp. 4124-4132.
- Park, K.J., Park, J.H., Park, J.H., Cho, E.B., Kim, B.J. & Kim, J.W. 2016, "The First Korean Family With Hereditary Gelsolin Amyloidosis Caused by p.D214Y Mutation in the GSN Gene", *Annals of laboratory medicine*, vol. 36, no. 3, pp. 259-262.
- Pasquali-Ronchetti, I. & Baccarani-Contrì, M. 1997, "Elastic fiber during development and aging", *Microscopy research and technique*, vol. 38, no. 4, pp. 428-435.
- Paunio, T., Kangas, H., Heinonen, O., Buc-Caron, M.H., Robert, J.J., Kaasinen, S., Julkunen, I., Mallet, J. & Peltonen, L. 1998, "Cells of the neuronal lineage play a major role in the generation of amyloid precursor fragments in gelsolin-related amyloidosis", *The Journal of biological chemistry*, vol. 273, no. 26, pp. 16319-16324.
- Paunio, T., Kangas, H., Kalkkinen, N., Haltia, M., Palo, J. & Peltonen, L. 1994, "Toward understanding the pathogenic mechanisms in gelsolin-related amyloidosis: in vitro expression reveals an abnormal gelsolin fragment", *Human molecular genetics*, vol. 3, no. 12, pp. 2223-2229.
- Paunio, T., Kangas, H., Kiuru, S., Palo, J., Peltonen, L. & Syvanen, A.C. 1997, "Tissue distribution and levels of gelsolin mRNA in normal individuals and patients with gelsolin-related amyloidosis", *FEBS letters*, vol. 406, no. 1-2, pp. 49-55.
- Paunio, T., Kiuru, S., Karonen, S., Palo, J. & Peltonen, L. 1994, "Quantification of serum and cerebrospinal fluid gelsolin in familial amyloidosis, Finnish type (AGel)", *Amyloid*, vol. 1, no. 2, pp. 80-89.



- Peng, S., Larsson, A., Wassberg, E., Gerwins, P., Thelin, S., Fu, X. & Westermark, P. 2007, "Role of aggregated medin in the pathogenesis of thoracic aortic aneurysm and dissection", *Laboratory investigation; a journal of technical methods and pathology*, vol. 87, no. 12, pp. 1195-1205.
- Pepys, M.B. 2018, "The Pentraxins 1975-2018: Serendipity, Diagnostics and Drugs", *Frontiers in immunology*, vol. 9, pp. 2382.
- Pepys, M.B., Baltz, M.L., de Beer, F.C., Dyck, R.F., Holford, S., Breathnach, S.M., Black, M.M., Tribe, C.R., Evans, D.J. & Feinstein, A. 1982, "Biology of serum amyloid P component", *Annals of the New York Academy of Sciences*, vol. 389, pp. 286-298.
- Pepys, M.B., Dyck, R.F., de Beer, F.C., Skinner, M. & Cohen, A.S. 1979, "Binding of serum amyloid P-component (SAP) by amyloid fibrils", *Clinical and experimental immunology*, vol. 38, no. 2, pp. 284-293.
- Pepys, M.B., Herbert, J., Hutchinson, W.L., Tennent, G.A., Lachmann, H.J., Gallimore, J.R., Lovat, L.B., Bartfai, T., Alanine, A., Hertel, C., Hoffmann, T., Jakob-Roetne, R., Norcross, R.D., Kemp, J.A., Yamamura, K., Suzuki, M., Taylor, G.W., Murray, S., Thompson, D., Purvis, A., Kolstoe, S., Wood, S.P. & Hawkins, P.N. 2002, "Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis", *Nature*, vol. 417, no. 6886, pp. 254-259.
- Perrin, B.J. & Ervasti, J.M. 2010, "The actin gene family: function follows isoform", *Cytoskeleton (Hoboken, N.J.)*, vol. 67, no. 10, pp. 630-634.
- Pezzini, A., Del Zotto, E., Volonghi, I., Giossi, A., Costa, P. & Padovani, A. 2009, "Cerebral amyloid angiopathy: a common cause of cerebral hemorrhage", *Current medicinal chemistry*, vol. 16, no. 20, pp. 2498-2513.
- Picken, M.M. 2007, "Immunoglobulin light and heavy chain amyloidosis AL/AH: renal pathology and differential diagnosis", *Contributions to nephrology*, vol. 153, pp. 135-155.
- Pihlmaa, T., Rautio, J., Kiuru-Enari, S. & Suominen, S. 2011, "Gelsolin amyloidosis as a cause of early aging and progressive bilateral facial paralysis", *Plastic and Reconstructive Surgery*, vol. 127, no. 6, pp. 2342-2351.
- Pihlmaa, T., Salmi, T., Suominen, S. & Kiuru-Enari, S. 2015, "Progressive cranial nerve involvement and grading of facial paralysis in gelsolin amyloidosis", *Muscle & nerve*, .
- Pihlmaa, T., Suominen, S., Kiuru-Enari, S. & Tanskanen, M. 2016, "Increasing amount of amyloid are associated with the severity of clinical features in hereditary gelsolin (AGel) amyloidosis", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 23, no. 4, pp. 225-233.
- Pitkanen, P., Westermark, P. & Cornwell, G.G., 3rd 1984, "Senile systemic amyloidosis", *The American journal of pathology*, vol. 117, no. 3, pp. 391-399.
- Polyakova, V., Loeffler, I., Hein, S., Miyagawa, S., Piotrowska, I., Dammer, S., Risteli, J., Schaper, J. & Kostin, S. 2011, "Fibrosis in endstage human heart failure: severe changes in collagen metabolism and MMP/TIMP profiles", *International journal of cardiology*, vol. 151, no. 1, pp. 18-33.
- Prusiner, S.B. 1998, "Prions", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 23, pp. 13363-13383.

- Puchtler, H., Sweat, F. & Levine, M. 1962, "On the binding of Congo red by amyloid", *Journal of Histochemistry and Cytochemistry*, vol. 10, pp. 355-364.
- Ratnaswamy, G., Koepf, E., Bekele, H., Yin, H. & Kelly, J.W. 1999, "The amyloidogenicity of gelsolin is controlled by proteolysis and pH", *Chemistry & biology*, vol. 6, no. 5, pp. 293-304.
- Rauz, S. & Saw, V.P. 2010, "Serum eye drops, amniotic membrane and limbal epithelial stem cells--tools in the treatment of ocular surface disease", *Cell and Tissue Banking*, vol. 11, no. 1, pp. 13-27.
- Real de Asua, D., Costa, R., Galvan, J.M., Filigheddu, M.T., Trujillo, D. & Cadinanos, J. 2014, "Systemic AA amyloidosis: epidemiology, diagnosis, and management", *Clinical epidemiology*, vol. 6, pp. 369-377.
- Ricard-Blum, S. 2011, "The collagen family", *Cold Spring Harbor perspectives in biology*, vol. 3, no. 1, pp. a004978.
- Robert, L., Robert, A.M. & Fulop, T. 2008, "Rapid increase in human life expectancy: will it soon be limited by the aging of elastin?", *Biogerontology*, vol. 9, no. 2, pp. 119-133.
- Rowczenio, D., Dogan, A., Theis, J.D., Vrana, J.A., Lachmann, H.J., Wechalekar, A.D., Gilbertson, J.A., Hunt, T., Gibbs, S.D., Sattianayagam, P.T., Pinney, J.H., Hawkins, P.N. & Gillmore, J.D. 2011, "Amyloidogenicity and clinical phenotype associated with five novel mutations in apolipoprotein A-I", *The American journal of pathology*, vol. 179, no. 4, pp. 1978-1987.
- Rubenstein, P.A. 1990, "The functional importance of multiple actin isoforms", *BioEssays : news and reviews in molecular, cellular and developmental biology*, vol. 12, no. 7, pp. 309-315.
- Santos-Briz, A., Canueto, J., Antunez, P., Bravo, J., Garcia-Sanz, R. & de Unamuno, P. 2010, "Primary cutaneous localized amyloid elastosis", *The American Journal of Dermatopathology*, vol. 32, no. 1, pp. 86-90.
- Schmidt, E.K., Atula, S., Tanskanen, M., Nikoskinen, T., Notkola, I.L. & Kiuru-Enari, S. 2016, "Causes of death and life span in Finnish gelsolin amyloidosis", *Annals of Medicine*, vol. 48, no. 5, pp. 352-358.
- Schmidt, E.K., Kiuru-Enari, S., Atula, S. & Tanskanen, M. 2019, "Amyloid in parenchymal organs in gelsolin (AGel) amyloidosis", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 26, no. 3, pp. 118-124.
- Selleck, M.J., Senthil, M. & Wall, N.R. 2017, "Making Meaningful Clinical Use of Biomarkers", *Biomarker insights*, vol. 12, pp. 1177271917715236.
- Selve, N. & Wegner, A. 1987, "pH-dependent rate of formation of the gelsolin-actin complex from gelsolin and monomeric actin", *European journal of biochemistry*, vol. 168, no. 1, pp. 111-115.
- Sepp, N., Pichler, E., Breathnach, S.M., Fritsch, P. & Hintner, H. 1990, "Amyloid elastosis: analysis of the role of amyloid P component", *Journal of the American Academy of Dermatology*, vol. 22, no. 1, pp. 27-34.

- Sethi, S., Dasari, S., Amin, M.S., Vrana, J.A., Theis, J.D., Alexander, M.P. & Kurtin, P.J. 2017, "Clinical, biopsy, and mass spectrometry findings of renal gelsolin amyloidosis", *Kidney international*, vol. 91, no. 4, pp. 964-971.
- Sethi, S., Theis, J.D., Quint, P., Maierhofer, W., Kurtin, P.J., Dogan, A. & Highsmith, E.W., Jr 2013, "Renal amyloidosis associated with a novel sequence variant of gelsolin", *American Journal of Kidney Diseases : The Official Journal of the National Kidney Foundation*, vol. 61, no. 1, pp. 161-166.
- Shekhonin, B.V., Domogatsky, S.P., Muzykantov, V.R., Idelson, G.L. & Rukosuev, V.S. 1985, "Distribution of type I, III, IV and V collagen in normal and atherosclerotic human arterial wall: immunomorphological characteristics", *Collagen and related research*, vol. 5, no. 4, pp. 355-368.
- Sherratt, M.J. 2009, "Tissue elasticity and the ageing elastic fibre", *Age (Dordrecht, Netherlands)*, vol. 31, no. 4, pp. 305-325.
- Shoja, M.M., Ardalan, M.R., Tubbs, R.S. & Kiuru-Enari, S. 2009, "Outcome of renal transplant in hereditary gelsolin amyloidosis", *The American Journal of the Medical Sciences*, vol. 337, no. 5, pp. 370-372.
- Silacci, P., Mazzolai, L., Gauci, C., Stergiopoulos, N., Yin, H.L. & Hayoz, D. 2004, "Gelsolin superfamily proteins: key regulators of cellular functions", *Cellular and molecular life sciences : CMLS*, vol. 61, no. 19-20, pp. 2614-2623.
- Sipe, J.D., Benson, M.D., Buxbaum, J.N., Ikeda, S.I., Merlini, G., Saraiva, M.J. & Westermark, P. 2016, "Amyloid fibril proteins and amyloidosis: chemical identification and clinical classification International Society of Amyloidosis 2016 Nomenclature Guidelines", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, vol. 23, no. 4, pp. 209-213.
- Sipe, J.D. & Cohen, A.S. 2000, "Review: history of the amyloid fibril", *Journal of structural biology*, vol. 130, no. 2-3, pp. 88-98.
- Snorraddottir, A.O., Isaksson, H.J., Ingthorsson, S., Olafsson, E., Palsdottir, A. & Bragason, B.T. 2017, "Pathological changes in basement membranes and dermal connective tissue of skin from patients with hereditary cystatin C amyloid angiopathy", *Laboratory investigation; a journal of technical methods and pathology*, vol. 97, no. 4, pp. 383-394.
- Solomon, J.P., Bourgault, S., Powers, E.T. & Kelly, J.W. 2011, "Heparin binds 8 kDa gelsolin cross-beta-sheet oligomers and accelerates amyloidogenesis by hastening fibril extension", *Biochemistry*, vol. 50, no. 13, pp. 2486-2498.
- Solomon, J.P., Page, L.J., Balch, W.E. & Kelly, J.W. 2012, "Gelsolin amyloidosis: genetics, biochemistry, pathology and possible strategies for therapeutic intervention", *Critical reviews in biochemistry and molecular biology*, vol. 47, no. 3, pp. 282-296.
- Solomon, J.P., Yonemoto, I.T., Murray, A.N., Price, J.L., Powers, E.T., Balch, W.E. & Kelly, J.W. 2009, "The 8 and 5 kDa fragments of plasma gelsolin form amyloid fibrils by a nucleated polymerization mechanism, while the 68 kDa fragment is not amyloidogenic", *Biochemistry*, vol. 48, no. 48, pp. 11370-11380.
- Soto, C., Castano, E.M., Prelli, F., Kumar, R.A. & Baumann, M. 1995, "Apolipoprotein E increases the fibrillogenic potential of synthetic peptides derived from Alzheimer's, gelsolin and AA amyloids", *FEBS letters*, vol. 371, no. 2, pp. 110-114.

- Spinardi, L. & Witke, W. 2007, "Gelsolin and diseases", *Sub-cellular biochemistry*, vol. 45, pp. 55-69.
- Srivastava, A., Arya, P., Goel, S., Kundu, B., Mishra, P. & Fnu, A. 2015, "Gelsolin Amyloidogenesis Is Effectively Modulated by Curcumin and Emetine Conjugated PLGA Nanoparticles", *PloS one*, vol. 10, no. 5, pp. e0127011.
- Steiner, I. & Hajkova, P. 2006, "Patterns of isolated atrial amyloid: a study of 100 hearts on autopsy", *Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology*, vol. 15, no. 5, pp. 287-290.
- Suk, J.Y., Zhang, F., Balch, W.E., Linhardt, R.J. & Kelly, J.W. 2006, "Heparin accelerates gelsolin amyloidogenesis", *Biochemistry*, vol. 45, no. 7, pp. 2234-2242.
- Sun, H.Q., Wooten, D.C., Janmey, P.A. & Yin, H.L. 1994, "The actin side-binding domain of gelsolin also caps actin filaments. Implications for actin filament severing", *The Journal of biological chemistry*, vol. 269, no. 13, pp. 9473-9479.
- Sun, H.Q., Yamamoto, M., Mejillano, M. & Yin, H.L. 1999, "Gelsolin, a multifunctional actin regulatory protein", *The Journal of biological chemistry*, vol. 274, no. 47, pp. 33179-33182.
- Suwabe, H., Serizawa, A., Kajiwar, H., Ohkido, M. & Tsutsumi, Y. 1999, "Degenerative processes of elastic fibers in sun-protected and sun-exposed skin: immunoelectron microscopic observation of elastin, fibrillin-1, amyloid P component, lysozyme and alpha1-antitrypsin", *Pathology international*, vol. 49, no. 5, pp. 391-402.
- Szabo, Z., Crepeau, M.W., Mitchell, A.L., Stephan, M.J., Puntel, R.A., Yin Loke, K., Kirk, R.C. & Urban, Z. 2006, "Aortic aneurysmal disease and cutis laxa caused by defects in the elastin gene", *Journal of medical genetics*, vol. 43, no. 3, pp. 255-258.
- Tanaka, J. & Sobue, K. 1994, "Localization and characterization of gelsolin in nervous tissues: gelsolin is specifically enriched in myelin-forming cells", *The Journal of neuroscience : the official journal of the Society for Neuroscience*, vol. 14, no. 3 Pt 1, pp. 1038-1052.
- Tanaka, M., Mullauer, L., Ogiso, Y., Fujita, H., Moriya, S., Furuuchi, K., Harabayashi, T., Shinohara, N., Koyanagi, T. & Kuzumaki, N. 1995, "Gelsolin: a candidate for suppressor of human bladder cancer", *Cancer research*, vol. 55, no. 15, pp. 3228-3232.
- Tanskanen, M., Paetau, A., Salonen, O., Salmi, T., Lamminen, A., Lindsberg, P., Somer, H. & Kiuru-Enari, S. 2009, "Severe ataxia with neuropathy in hereditary gelsolin amyloidosis", *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*, , pp. 1-7.
- Tian, J., Shi, J. & Mann, D.M. 2004, "Cerebral amyloid angiopathy and dementia", *Panminerva medica*, vol. 46, no. 4, pp. 253-264.
- Valleix, S., Gillmore, J.D., Bridoux, F., Mangione, P.P., Dogan, A., Nedelec, B., Boimard, M., Touchard, G., Goujon, J.M., Lacombe, C., Lozeron, P., Adams, D., Lacroix, C., Maisonnobe, T., Plante-Bordeneuve, V., Vrana, J.A., Theis, J.D., Giorgetti, S., Porcari, R., Ricagno, S., Bolognesi, M., Stoppini, M., Delpech, M., Pepys, M.B., Hawkins, P.N. & Bellotti, V. 2012, "Hereditary systemic amyloidosis due to Asp76Asn variant beta2-microglobulin", *The New England journal of medicine*, vol. 366, no. 24, pp. 2276-2283.

- Van Overbeke, W., Verhelle, A., Everaert, I., Zwaenepoel, O., Vandekerckhove, J., Cuvelier, C., Derave, W. & Gettemans, J. 2014, "Chaperone nanobodies protect gelsolin against MT1-MMP degradation and alleviate amyloid burden in the gelsolin amyloidosis mouse model", *Molecular therapy : the journal of the American Society of Gene Therapy*, vol. 22, no. 10, pp. 1768-1778.
- Van Overbeke, W., Wongsantichon, J., Everaert, I., Verhelle, A., Zwaenepoel, O., Loonchanta, A., Burtnick, L.D., De Ganck, A., Hocheplied, T., Haigh, J., Cuvelier, C., Derave, W., Robinson, R.C. & Gettemans, J. 2015, "An ER-directed gelsolin nanobody targets the first step in amyloid formation in a gelsolin amyloidosis mouse model", *Human molecular genetics*, vol. 24, no. 9, pp. 2492-2507.
- Vandekerckhove, J. & Weber, K. 1979, "The complete amino acid sequence of actins from bovine aorta, bovine heart, bovine fast skeletal muscle, and rabbit slow skeletal muscle. A protein-chemical analysis of muscle actin differentiation", *Differentiation; research in biological diversity*, vol. 14, no. 3, pp. 123-133.
- Vasconcellos, C.A. & Lind, S.E. 1993, "Coordinated inhibition of actin-induced platelet aggregation by plasma gelsolin and vitamin D-binding protein", *Blood*, vol. 82, no. 12, pp. 3648-3657.
- Vecchietti, G., Masouye, I., Salomon, D., Dozier, C., Saurat, J.H., Helg, C. & Borradori, L. 2003, "An unusual form of primary systemic amyloidosis: amyloid elastosis: report of a case treated by haematopoietic cell transplantation", *The British journal of dermatology*, vol. 148, no. 1, pp. 154-159.
- Verbeek, M.M., de Waal, R.M. & Vinters, H.V. (eds) 2013, *Cerebral Amyloid Angiopathy in Alzheimer's Disease and Related Disorders*, Springer Science & Business Media, Dordrecht.
- Verhelle, A., Nair, N., Everaert, I., Van Overbeke, W., Supply, L., Zwaenepoel, O., Peleman, C., Van Dorpe, J., Lahoutte, T., Devoogdt, N., Derave, W., Chuah, M.K., Vanden Driessche, T. & Gettemans, J. 2017, "AAV9 delivered bispecific nanobody attenuates amyloid burden in the gelsolin amyloidosis mouse model", *Human molecular genetics*, vol. 26, no. 7, pp. 1353-1364.
- Vidal, R., Barbeito, A.G., Miravalle, L. & Ghetti, B. 2009, "Cerebral amyloid angiopathy and parenchymal amyloid deposition in transgenic mice expressing the Danish mutant form of human BRI2", *Brain pathology (Zurich, Switzerland)*, vol. 19, no. 1, pp. 58-68.
- Vidal, R., Frangione, B., Rostagno, A., Mead, S., Revesz, T., Plant, G. & Ghiso, J. 1999, "A stop-codon mutation in the BRI gene associated with familial British dementia", *Nature*, vol. 399, no. 6738, pp. 776-781.
- Vora, M., Kevil, C.G. & Herrera, G.A. 2017, "Contribution of human smooth muscle cells to amyloid angiopathy in AL (light-chain) amyloidosis", *Ultrastructural pathology*, vol. 41, no. 5, pp. 358-368.
- Vouyiouklis D.A. & Brophy P.J. 1997, "A novel gelsolin isoform expressed by oligodendrocytes in the central nervous system", *Journal of Neurochemistry*, vol. 69, no. 3, pp. 995-1005.
- Wang, Y., Feng, X., Shen, B., Ma, J. & Zhao, W. 2017, "Is Vascular Amyloidosis Intertwined with Arterial Aging, Hypertension and Atherosclerosis?", *Frontiers in genetics*, vol. 8, pp. 126.
- Way, M., Gooch, J., Pope, B. & Weeds, A.G. 1989, "Expression of human plasma gelsolin in *Escherichia coli* and dissection of actin binding sites by segmental deletion mutagenesis", *The Journal of cell biology*, vol. 109, no. 2, pp. 593-605.

- Weeds, A.G., Gooch, J., McLaughlin, P. & Maury, C.P. 1993, "Variant plasma gelsolin responsible for familial amyloidosis (Finnish type) has defective actin severing activity", *FEBS letters*, vol. 335, no. 1, pp. 119-123.
- Weiss, R., Lifshitz, V. & Frenkel, D. 2011, "TGF-beta1 affects endothelial cell interaction with macrophages and T cells leading to the development of cerebrovascular amyloidosis", *Brain, behavior, and immunity*, vol. 25, no. 5, pp. 1017-1024.
- Wen, D., Corina, K., Chow, E.P., Miller, S., Janmey, P.A. & Pepinsky, R.B. 1996, "The plasma and cytoplasmic forms of human gelsolin differ in disulfide structure", *Biochemistry*, vol. 35, no. 30, pp. 9700-9709.
- Werb, Z., Banda, M.J., McKerrow, J.H. & Sandhaus, R.A. 1982, "Elastases and elastin degradation", *The Journal of investigative dermatology*, vol. 79 Suppl 1, pp. 154s-159s.
- Westberg, J.A., Zhang, K.Z. & Andersson, L.C. 1999, "Regulation of neural differentiation by normal and mutant (G654A, amyloidogenic) gelsolin", *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, vol. 13, no. 12, pp. 1621-1626.
- Westermarck, G.T., Fandrich, M. & Westermarck, P. 2015, "AA amyloidosis: pathogenesis and targeted therapy", *Annual review of pathology*, vol. 10, pp. 321-344.
- Wilkins, J.A., Schwartz, J.H. & Harris, D.A. 1983, "Brevin, a serum protein that acts on the barbed end of actin filaments", *Cell biology international reports*, vol. 7, no. 12, pp. 1097-1104.
- Winkelmann, R.K., Peters, M.S. & Venencie, P.Y. 1985, "Amyloid elastosis. A new cutaneous and systemic pattern of amyloidosis", *Archives of Dermatology*, vol. 121, no. 4, pp. 498-502.
- Wisniewski, H.M., Frackowiak, J. & Mazur-Kolecka, B. 1995, "In vitro production of beta-amyloid in smooth muscle cells isolated from amyloid angiopathy-affected vessels", *Neuroscience letters*, vol. 183, no. 1-2, pp. 120-123.
- Witke, W., Sharpe, A.H., Hartwig, J.H., Azuma, T., Stossel, T.P. & Kwiatkowski, D.J. 1995, "Hemostatic, inflammatory, and fibroblast responses are blunted in mice lacking gelsolin", *Cell*, vol. 81, no. 1, pp. 41-51.
- Xu, J. & Shi, G.P. 2014, "Vascular wall extracellular matrix proteins and vascular diseases", *Biochimica et biophysica acta*, vol. 1842, no. 11, pp. 2106-2119.
- Xue, W.F., Homans, S.W. & Radford, S.E. 2009, "Amyloid fibril length distribution quantified by atomic force microscopy single-particle image analysis", *Protein engineering, design & selection : PEDS*, vol. 22, no. 8, pp. 489-496.
- Yamanaka, S., Miyazaki, Y., Kasai, K., Ikeda, S., Kiuru-Enari, S. & Hosoya, T. 2013, "Hereditary renal amyloidosis caused by a heterozygous G654A gelsolin mutation: a report of two cases", *Clinical kidney journal*, vol. 6, no. 2, pp. 189-193.
- Yamashita, T., Ando, Y., Katsuragi, S., Nakamura, M., Obayashi, K., Haraoka, K., Ueda, M., Xuguo, S., Okamoto, S. & Uchino, M. 2005, "Muscular amyloid angiopathy with amyloidogenic transthyretin Ser50Ile and Tyr114Cys", *Muscle & nerve*, vol. 31, no. 1, pp. 41-45.

- Yin, H.L., Albrecht, J.H. & Fattoum, A. 1981, "Identification of gelsolin, a  $\text{Ca}^{2+}$ -dependent regulatory protein of actin gel-sol transformation, and its intracellular distribution in a variety of cells and tissues", *The Journal of cell biology*, vol. 91, no. 3 Pt 1, pp. 901-906.
- Yin, H.L., Hartwig, J.H., Maruyama, K. & Stossel, T.P. 1981, " $\text{Ca}^{2+}$  control of actin filament length. Effects of macrophage gelsolin on actin polymerization", *The Journal of biological chemistry*, vol. 256, no. 18, pp. 9693-9697.
- Yin, H.L., Kwiatkowski, D.J., Mole, J.E. & Cole, F.S. 1984, "Structure and biosynthesis of cytoplasmic and secreted variants of gelsolin", *The Journal of biological chemistry*, vol. 259, no. 8, pp. 5271-5276.
- Yin, H.L. & Stossel, T.P. 1979, "Control of cytoplasmic actin gel-sol transformation by gelsolin, a calcium-dependent regulatory protein", *Nature*, vol. 281, no. 5732, pp. 583-586.
- Yin, H.L., Zaner, K.S. & Stossel, T.P. 1980, " $\text{Ca}^{2+}$  control of actin gelation. Interaction of gelsolin with actin filaments and regulation of actin gelation", *The Journal of biological chemistry*, vol. 255, no. 19, pp. 9494-9500.
- Yu, J.C. & Gotlieb, A.I. 1992, "Disruption of endothelial actin microfilaments by protein kinase C inhibitors", *Microvascular research*, vol. 43, no. 1, pp. 100-111.
- Zorgati, H., Larsson, M., Ren, W., Sim, A.Y.L., Gettemans, J., Grimes, J.M., Li, W. & Robinson, R.C. 2019, "The role of gelsolin domain 3 in familial amyloidosis (Finnish type)", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 116, no. 28, pp. 13958-13963.